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Production of *Chironomus*, *Procladius*, and *Chaoborus* at Different Levels of Phytoplankton Biomass in Lake Memphremagog, Quebec–Vermont[1]

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DERMOTT, R. M., J. KALFF, W. C. LEGGETT, AND J. SPENCE. 1977. Production of *Chironomus*, *Procladius*, and *Chaoborus* at different levels of phytoplankton biomass in Lake Memphremagog, Quebec–Vermont. J. Fish. Res. Board Can. 34: 2001–2007.

In Lake Memphremagog a north–south gradient in algal production and biomass exists during the ice-free period, with highest production in the southern areas. In response, the mean annual benthic standing stock (dry weight) in the south basin was 2.8 times greater than in the north basin at comparable depth. Mean annual standing stock of *Procladius denticulatus* and *Chironomus anthracinus* were significantly (5.8 and 3.1 times, respectively) higher in the south, while that of *Chaoborus punctipennus* did not differ significantly between areas. Annual production of *P. denticulatus* was greater in the south (0.861 g/m²) than in the north (0.143 g/m²). Similarly for *Chironomus anthracinus* annual production was greater in the south (3.393 g/m²) than in the north (1.264 g/m²). Production of *Chaoborus punctipennis* was greater in the north (0.066 g/m²) than in the south (0.348 g/m²). P:B ratios were less variable.

Key words: Lake Memphremagog, benthos, production, nutrient gradient, mortality

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Il existe dans le lac Memphrémagog un gradient nord–sud dans la production et la biomasse algales durant la période d'absence de glace, la production maximale étant dans les régions méridionales. En réponse, la biomasse benthique annuelle moyenne (poids sec) dans le bassin méridional est 2.8 fois supérieure à celle du bassin septentrional, à une profondeur comparable. La biomasse annuelle moyenne de *Procladius denticulatus* et de *Chironomus anthracinus* est

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significativement (5.8 et 3.1 fois respectivement) plus élevée au sud, alors que celle de *Chaoborus punctipennis* ne diffère pas notablement d'une région à l'autre. La production annuelle de *P. denticulatus* et de *Chironomus anthracinus* est plus forte dans le sud que dans le nord (0.861/0.143 et 3.393/1.264 g/m² respectivement). La production de *Chaoborus punctipennis* est maximale au nord (0.066/0.348 g/m²). Les taux de renouvellement sont moins variables.

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THE composition of lacustrine benthic communities is known to be influenced by the quantity of allochthonous and autochthonous matter available to them as food (Schneider et al. 1969; Johnson and Matheson 1968). Artificial (Smith 1969; Le Brasseur and Kennedy 1972) and cultural (Johnson and Brinkhurst 1971) nutrient enrichment of lakes have resulted in increased benthic biomass and production following increases in primary production and sedimentation rates. Benthic communities play an important role in the nutrition of higher trophic levels by linking them energetically with primary producers (Ball and Hayne 1952; Lellak 1965). Therefore, there should be a direct link between nutrient loading and production at all trophic levels.

The Lake Memphremagog Study, initiated in 1972, seeks to quantify the response of communities occupying various trophic levels to the pronounced nutrient gradient that occurs along the long axis of the lake. Associated with this nutrient gradient is a gradient in primary production, with summer annual phytoplankton biomass in the nutrient-rich south basin being 1.6 times greater than in the north (Fig. 1) (Ross and Kalff 1975). Fish biomass is also 2–3 times higher in the south basin than in the north (Nakashima and Leggett 1975; Gascon and Leggett 1977) and the species diversity of littoral zone fishes varies along the gradient (Nakashima et al. 1977). In this investigation the production and biomass of *Chironomus*, *Procladius*, and *Chaoborus* species, the dominant elements in the benthos of Lake Memphremagog, were examined for their relationship to prevailing levels of nutrients and primary production along the gradient on the one hand, and for their contribution to production at higher trophic levels on the other.

Materials and Methods

Sampling stations were established in the north and south basins of the lake (Fig. 1) in areas having comparable depths and substrates. The north basin site was situated 34 km from the inflow at Newport, Vermont, at a depth of 11.5 m. The south basin site was located 5 km from Newport at a depth of 9.5 m.

Between July 1972 and October 1973 triplicate

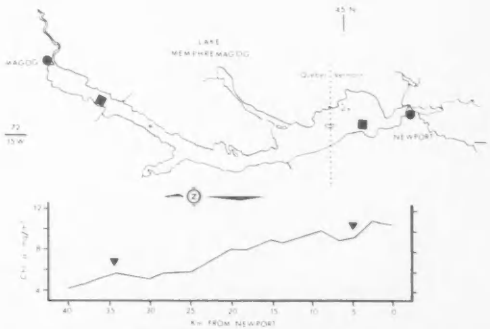

FIG. 1. Lake Memphremagog showing locations of sampling areas in the lake (■) and the algal biomass gradient (▼) as it appeared on August 3, 1973.

samples were collected at each site on a weekly basis during the summer, fortnightly in the autumn, and when possible during the winter months. Exploratory sampling was conducted at other areas in the two basins throughout the study to evaluate the generality of the findings at the main study locations. A tall (23 cm) Ekman sampler (sampling area 256 cm²) was used to obtain samples of the benthos, and only samples that had penetrated at least 9 cm into the substrate were used. All samples were screened (mesh size 0.24 mm) within 24 h of collection. The samples were refrigerated (<10°C) between collection and screening.

One sample from each date was subdivided into various taxonomical groupings and these were analyzed for mean lengths and weights of fresh organisms. Body length was measured with an ocular micrometer to the nearest 0.1 mm, head capsule length to the nearest 0.02 mm. These fresh specimens were then dried for 24 h at 60°C and weighed with an electrobalance. The two remaining replicate samples were preserved in 10% neutral formalin for later analysis of numerical composition, age structure, and growth.

Jónasson (1955) found a 50% loss of stage II instars of *Chironomus anthracinus* (head width — 0.210 mm), when using a 0.26-mm mesh screen. Calculations of total numbers of this instar of *C. anthracinus* were thus doubled to correct for losses of these smaller larvae during screening.

Water temperature and dissolved oxygen were measured at the sampling sites of each sampling date. Oxygen was measured with a Y.S.I. oxygen meter in 1972, and with a modified micro Winkler (Using

0.01 N sodium thiosulfate and 50-ml samples, Ross and Kalff 1975) during 1973.

Three major species of Diptera (*Procladius denticulatus*, *C. anthracinus*, and *Chaoborus punctipennis*), each having a 1-yr generation time and a discrete emergence period in Lake Memphremagog, were studied in detail.

Production of each of the species was calculated using the equation

$$P = \sum_{t=0}^{t} (\overline{W}t + 1 - \overline{W}t) \times \frac{Nt + Nt + 1}{2}$$

where N = number of specimens, W = mean weight, t = time of sampling (Edmondson and Winberg 1971; Jónasson 1972).

Mortality (M), an estimate of the weight lost from the population through various causes, was calculated as:

$$M = \sum_{t=0}^{t} (Nt - Nt + 1) \times \frac{\overline{W}t + 1 + \overline{W}t}{2}$$

Production during the sampling interval and mean interval standing stock was used to calculate the P:B ratio expressed as production of standing stock per day (Edmondson and Winberg 1971).

Annual production and P:B ratios could be calculated only for the generations studied from summer 1972 to emergence 1973. Seasonal values were obtained for the 1973 generation from the July appearance of young larvae to October 1973 when the study was terminated.

Results

Temperature and Oxygen Conditions

Water temperatures at the two sampling sites were similar, generally being within 0.5 deg C of one another throughout the study. In the south basin the thermocline penetrated to the bottom on July 25, 1972 and July 20, 1973. The water over the south station was thus stratified only during the early summer. At the north station the summer thermocline remained approximately 0.5 m above the sediment at the sampling site at a depth of 11 m until late August.

Dissolved oxygen levels averaged approximately 2 mg·ℓ^{-1} lower at the southern station than at the northern throughout July and August in spite of the late summer destratification in the south. The minimum oxygen level recorded 1 m above the sediments in the south was 1.9 mg·ℓ^{-1}. In the north the minimum level recorded was 2.6 mg·ℓ^{-1}. Oxygen levels below 1.0 mg·ℓ^{-1} were common at the sediment–water interface at the south basin station during July and August. Such low values could cause localized stagnation in the zone of microstratification at the mud–water interface.

Benthic Biomass

The mean annual total benthic standing stock (dry weight) at the south basin site (3.36 ± 0.25 g/m^2) was 2.8 times higher than that of the north basin site (1.19 ± 0.06 g/m^2) in 1972 and 1973 ($t = 19.5$, df = 42, $P < 0.01$). The percent composition of the fauna at both sites was similar, the dominant elements being, in order of numerical abundance, *Tanytarsus* spp., *Procladius* spp., *Chironomus anthracinus*, and *Chaoborus punctipennis*. The mean standing stock of *P. denticulatus* and *Chironomus anthracinus* over the same period was, respectively, 5.8 ($t = 2.57$, df = 51, $P < 0.05$) and 3.1 ($t = 3.94$, df = 46, $P < 0.01$) times higher in the south than in the north (Table 1). The mean biomass of *Chaoborus punctipennis* was 2.8 times higher in the north; however, the difference was not significant ($t = 1.02$, df = 52, $P > 0.05$).

The cooler, deeper profoundal zone of the north basin, offshore from the main north basin sampling site, supported a fauna consisting mainly of *Phaenospectra* sp., *Microspectra* sp., *Pontoporeia hoyi* which were not found in the south basin.

Growth and Production

Chironomus anthracinus — The large size of the detritivore *C. anthracinus*, relative to other elements of the benthic biota, resulted in its being the dominant benthic species, on a weight basis, in waters <20 m deep.

Daily growth of *C. anthracinus* was most rapid during autumn. This was followed by a near cessation of growth during winter (Fig. 2). Growth in the south was slightly greater than in the north, the larvae of the south basin being significantly larger in both length and dry weight. The greatest weight difference occurred during winter 1972–73 when the mean larval weight (2.46 mg) in the south basin was 170% greater than that in the north basin.

Seasonal variation in daily growth was also greater in the south basin (Fig. 2). Growth in the south basin was retarded in August 1973 relative to August 1972, to the extent that mean weight of larvae in the north basin in early September briefly surpassed mean weight of those in the south. The very low oxygen levels at the mud–water interface in the south basin during this period may have been responsible for this slower growth. Following turnover in September and the resultant increase in O_2 levels, growth in the south basin resumed a higher rate and by October 1973 the mean larval weight in the south basin had surpassed that in the north basin. The

TABLE 1. Annual mean standing stock (SD) (dry weight) production, P:B, and mortality of three benthic invertebrates (*Chironomus anthracinus*, *Procladius denticulatus*, and *Chaoborus punctipennis*) at northern (N) and southern (S) sampling sites, Lake Memphremagog.

		C. anthracinus		*P. denticulatus*		*C. punctipennis*	
		N	S	N	S	N	S
Standing stock (g/m^2)	1972–73	0.352(0.169)	1.092(0.793)	0.053(0.026)	0.306(0.224)	0.097(0.081)	0.035(0.018)
Production (g/m^2)	1972	1.264	3.393	0.143	0.861	0.348	0.066
	1973	0.249	1.122	—	—	0.262	0.139
(P:B/day)	1972	0.009	0.010	0.008	0.008	0.010	0.005
	1973	0.039	0.041	—	—	0.042	0.012
Mortality (g/m^2)	1972	0.740	2.580	0.096	0.398	0.529	0.147
	1973	0.215	0.485	—	—	0.285	0.302

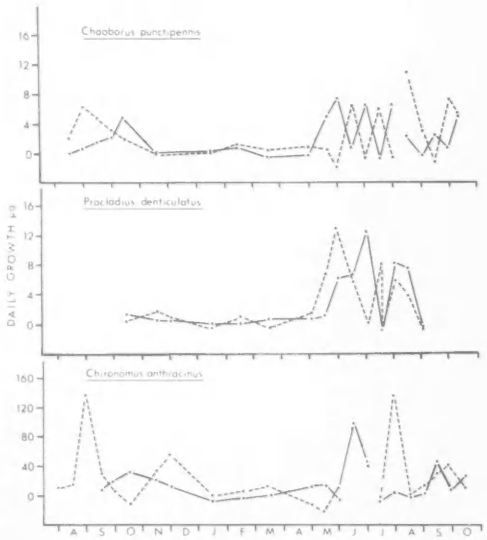

FIG. 2. Daily growth rates for larvae of three Diptera species sampled at south (- - - - -) and north (——) basin stations in Lake Memphremagog, 1972–73. Breaks in lines indicate emergence period.

mean dry weights of the larvae of *C. anthracinus* in the two basins over this period were (NS — not significant):

Aug. 6	III instar	0.026 mg	0.049 mg	$P < 0.01$
Sept. 2	IV instar	0.390	0.326	NS
Sept. 23	IV instar	0.641	1.068	$P < 0.001$

Annual production by the 1972 generation was 2.7 times higher in the south basin than in the north from August 1972 to July 1973. Seasonal production in 1973 (approximately 100 days) was 4.5 times higher in the south. Mortalities, or lost production, over the same time intervals were 3.5 and 2.2 times higher in the south, respectively. Daily P:B ratios did not differ between the two sites (Table 1).

Procladius denticulatus — The daily growth of *P. denticulatus* was similar in the two basins (Fig. 2). Growth was limited during winter, increasing dramatically in late May when the bottom water temperature reached 8°C and molting from the III–IV instar occurred. Following this, mean larval weight increased rapidly.

Production estimates for this species were possible only for the 1972 year-class because small II instar larvae did not appear in the samples until mid-September. Annual production by the 1972 year-class (October 1972–July 1973) was 6.0 times higher in the south basin than in the north. Mortality during the same interval was 4.1 times higher in the south. Daily P:B ratios were identical at the two sites (Table 1).

Chaoborus punctipennis — *Chaoborus punctipennis* occurred in greatest numbers in the deeper portions of the north basin at approximately 20 m deep where the maximum population recorded was $2480/m^2$. The average abundance at both the north and south basin sites was approximately $200/m^2$.

The daily growth rate (Fig. 2) of *C. punctipennis* was similar at both sampling sites throughout the study period with one exception during August 1973 when recruitment of smaller II instar larvae in the north basin reduced the mean weight of individuals in the population relative to that in the south. The growth of *C. punctipennis* ceased over winter, resuming again during spring.

The net annual production of the 1972 year-class (August 1972–August 1973) was 5.3 times higher in the north basin than in the south. The seasonal estimate for the 1973 year-class (September–October 1973) exhibited a similar trend, being 1.9 times higher in the north. In 1972 mortality was 3.6 times higher in the north; in 1973 it was approximately equal in the two basins. Daily P:B ratios were 2 and 4 times higher in the north in 1972 and 1973, respectively (Table 1).

TABLE 2. Comparison of benthic production levels (kg·ha^{-1} dry weight) in Lake Memphremagog with other lakes reported in the literature.

	Lake Memphremagog[a] 1972–73		Lake Esrom[b]		Lake Beloie[c]	Linsley Pond[c]	Lake Mendota[c]	Lake Nipigon[c]	Lake Ontario Bay of Quinte[d]	
	South Basin	North Basin	Directly	From mean kcal·m^{-1}					Glenora	Conway
Chironomus	33.93	12.64	139–210	128.9	26.8	44.8	20.2	0.4	17.41	0.920
Oligochaeta	—	—	—	11.6	29.0	7.2	6.1	0.5	17.77	0.643
Amphipoda	—	4.3	—	—	—	—	—	1.5	—	11.44
Mollusca	—	—	—	4.4	—	—	3.8	2.7	2.24	1.04
Tanypodinae (*Procladius*)	8.61	1.43	4.5	5.2	2.8	—	1.2	—	—	—
Chaoborus	0.66	3.48	2.7–26.5	29.2	17.6	19.8	23.2	—	—	—

[a]Present study; [b]from Jónasson (1972); [c]from Frey (1963); [d]from Johnson and Brinkhurst (1971).

Discussion

Since depth, substrate, and temperature conditions at the two study sites were similar, the 2.8 times increase in benthic biomass in the south, relative to the north, is the indirect result of higher nutrient concentrations in the south basin (Kalff and Leggett 1975), and the direct result of the higher algal standing stocks (1.6:1) and production (1.25:1) there (Ross and Kalff 1975). While the two basins differ in morphology, the north basin having a greater depth, the areas we studied are representative of the littoral and sublittoral zones (100% of basin area, 4400 ha in south; 55% of basin area, 1050 ha in north) of their respective basins (Nakashima and Leggett 1975). Le Brasseur and Kennedy (1972) and Smith (1969) also reported that increased phytoplankton levels in lakes (in these cases resulting from experimental additions of nutrient salts) led to increases in the consumer biomass.

This higher benthic standing stock in the south, attributable primarily to the 2.7 times increase in annual production and 2.8 times increase in standing stock of *Chironomus* spp., contributes in turn to increased fish standing stock in the south. Nakashima and Leggett (1975) reported the standing stock of perch in waters <12 m deep to be 2.5 times greater in the south basin than the north. Similarly, Gascon and Leggett (1977) found the standing stock of littoral zone fishes in Lake Memphremagog to be approximately 3 times greater in the south basin of the lake than in the north. In both cases chironomid larvae and pupae were the dominant items in the diet of carnivorous and omnivorous fishes <250 mm long, which constituted the great majority of the specimens captured in 4 yr of study. In addition, the intensity of benthic vs. pelagic feeding by littoral zone fishes was greatly increased in the south (Gascon and Leggett 1977). This increased standing stock of fish in the south basin may contribute, through increased predation, to the increased mortality of the two larger species (*Chironomus anthracinus* and *Chaoborus punctipennis*) in the south basin.

The benthic production of the two basins of Lake Memphremagog compares favorably with that observed in other studies (Table 2). The south basin values, which are representative of the entire basin, are characteristic of those observed in other enriched (eutrophic) environments such as Lake Beloie, Linsley Pond, Lake Mendota, and the Bay of Quinte near Glenora. The north basin values, which are biased upward because they exclude the less productive profoundal zone (45% of basin area), are more representative of the mesotrophic conditions prevailing in the north basin (Ross and Kalff 1975).

At the level of individual species, the consumer *Chironomus anthracinus* displayed major peaks of growth that corresponded with, or closely followed, the late summer and autumn peaks in algal standing stock (Ross and Kalff 1975). Apart from these summer peaks the highest growth rates occurred in September 1972 and 1973 immediately following the molt to the last instar. This pattern of maximum growth rates following the molt to the IV instar was also observed in *P. denticulatus* and *Chaoborus punctipennis*. The period of maximum growth in the three species thus depends, to a large extent, on the particular life history characteristic of the population. *Chironomus anthracinus* and *Chaoborus punctipennis*, which molt to the IV instar stage in late August and September, exhibited maximum growth at that time while *P. denticulatus*, which overwinters in the III instar, experienced maximum growth in June immediately following the final molt. Koskinen (1969) reported a similar pattern in *Chironomus salinarius* in Raungfjord near Bergen, Norway. There the larvae overwinter in the III and IV instars so that molting into the final instar takes place in August and June. Under these conditions maximum growth rates occurred in June and August and were primarily due to the rapid growth of the final instar stage.

Growth also appears to have been influenced by environmental conditions. This was evident in the depressed growth of *C. anthracinus* in the south basin in August 1973 during a period of low oxygen levels at the mud–water interface. Jónasson (1972), working in Lake Esrom, found no evidence of summer peaks in the growth of *C. anthracinus*. There maximum growth coincided with the spring and autumn overturns, summer and winter growth being depressed by oxygen depletion and winter temperatures, respectively.

In the south basin of Lake Memphremagog growth of *C. anthracinus* was accelerated relative to the north basin. This accelerated growth, rather than an increase in absolute abundance, was primarily responsible for the 3.1 times increase in total biomass and 2.7 times increase in annual production in the south basin relative to the north.

Procladius denticulatus, in contrast, exhibited similar growth in both basins. In this species growth was more closely linked to the attainment of suitable water temperatures in the spring and to the timing of the molt to the final instar than to seasonal or annual differences in food availability. The 6.0 times increase in annual production and a 5.8 times increase in biomass in the south basin, relative to the north was achieved by corresponding increase in relative abundance in the south basin.

Chaoborus punctipennis displayed both higher net production and standing stocks in the less enriched north basin, a reversal of the trend observed in *Procladius* and *Chironomus*. Egglinton (1931) and Deevey (1941) found that *Chaoborus* larvae are generally most abundant in the deeper areas of lakes up to 30 m in depth. Differences in mean depths between basins (south 6.9 m, north 13.5 m) probably account for this trend. We do not believe that differences in basin productivity are responsible since Stahl (1969) reports *Chaoborus* larvae are very tolerant of eutrophic conditions and are usually abundant in lakes too rich for *Chironomus* or *Procladius*.

Daily P:B ratios, a measure of production efficiency, did not differ significantly between basins for the two species exhibiting increased biomass and production in the more productive south basins, in spite of the differences in the availability of food there. In Lake Memphremagog this appears to be true not only for the benthos but also for the algal and fish communities. Ross and Kalff (1975) reported algal specific production, and the specific efficiency of production was similar in the north and south basins in spite of the approximate 2 times increase in total algal standing stock in the south basin. Moreover, Nakashima and Leggett (1975) found no differences in growth rates of yellow perch in the two basins in spite of the increased food availability there. The 2.5 times increase in perch standing stock per unit area observed in the south basin resulted from a corresponding increase in numbers per unit area. Johnson and Brinkhurst (1971) and Kalff and Welch (1974) have previously reported that growth efficiencies of algal and benthic communities remain stable or decrease under enriched conditions. The response of the algal, benthic, and fish communities of Lake Memphremagog to increased nutrient loading support these findings. The stability, or possible decline, of production efficiency under conditions of increased food supply apparently results from an inverse relationship between assimilation efficiency and food supply (Johnson and Brinkhurst 1971). This may explain, in large part, the relative stability of individual growth under conditions of enriched food supply and the resultant balancing of standing stock at one trophic level against resources at lower levels through changes in population size observed both in this study and by Kalff and Welch (1974), Moore (1941), Ganf and Viner (1973), and Nakashima and Legett (1975).

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Evidence for the Presence of a Metamorphic Check in Capelin (*Mallotus villosus*) Otoliths and Implications for Age Determination[1]

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Bailey, R. F. J., K. W. Able, and W. C. Leggett. 1977. Evidence for the presence of a metamorphic check in capelin (*Mallotus villosus*) otoliths and implications for age determination. J. Fish. Res. Board Can. 34: 2008–2014.

The otolith method of aging capelin (*Mallotus villosus*) from the estuary and western Gulf of St. Lawrence was validated. Hyaline zones are deposited annually in the late fall and winter and thus can be used in age determination. Back-calculated mean length at the first hyaline zone corresponded well with the average length of capelin larvae collected in November and this zone is interpreted as the first annulus. The mean size at the second hyaline zone corresponded exactly with the length at which capelin undergo a profound metamorphosis. This second hyaline zone, which is laid down in the summer of the 2nd yr, was thus interpreted to be a metamorphic check. The third hyaline zone, deposited at a mean length of 95 mm, which corresponded with the peak in the length frequency distribution (when allowance was made for growth), was interpreted as the second annulus. Analysis of two otolith aging techniques currently used suggests that the "Newfoundland" method misinterprets the metamorphic check as the first annulus, rejecting the true first annulus as a "larval check," a feature having no biological basis in fact. This results in correct ages, but estimates of lengths at the first annulus are exaggerated. The "Greenland" or "Danish" method may misinterpret the metamorphic check as the second annulus thereby resulting in erroneous ages.

Key words: capelin, *Mallotus villosus*, otoliths, age verification, Gulf of St. Lawrence

Bailey, R. F. J., K. W. Able, and W. C. Leggett. 1977. Evidence for the presence of a metamorphic check in capelin (*Mallotus villosus*) otoliths and implications for age determination. J. Fish. Res. Board Can. 34: 2008–2014.

Nous démontrons la validité d'une méthode de détermination, par les otolithes, de l'âge du capelan (*Mallotus villosus*) de l'estuaire et du golfe Saint-Laurent occidental. Les zones hyalines sont déposées annuellement à la fin de l'automne et en hiver, et peuvent donc être utilisées dans la détermination de l'âge. La longueur moyenne à la première zone hyaline, déterminée par rétrocalculs, correspond bien à la longueur moyenne des larves de capelan recueillies en novembre. Nous interprétons cette zone comme étant le premier annulus. La longueur moyenne à la deuxième zone hyaline correspond exactement à la longueur à laquelle le capelan subit une profonde métamorphose. Nous considérons donc la deuxième zone hyaline, formée au cours de l'été de la 2_e a, comme étant une marque métamorphique. Nous interprétons comme second annulus la troisième zone hyaline, déposée à une longueur moyenne de 95 mm, qui correspond au pic observé dans la distribution des fréquences de longueur (compte tenu de la croissance). L'analyse de deux méthodes de détermination de l'âge par les otolithes couramment utilisées suggère que la méthode de « Terre-Neuve » considère à tort la marque métamorphique comme premier annulus, écartant le véritable premier annulus comme « marque larvaire », un caractère en fait dépourvu de fondement biologique. Il en résulte des âges exacts, mais les estimés de longueurs au premier annulus sont exagérés. La méthode « grœnlandaise » ou « danoise » peut interpréter à tort la marque métamorphique et la considérer comme deuxième annulus, donnant ainsi des âges inexacts.

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The development of a large international fishery for capelin in the Canadian Atlantic since 1972 (Winters 1975) has resulted in a proportionate increase in the demand for biological data relevant to the effective management of the resource. Central to the development of such data is the

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accurate determination of ages. Otoliths have traditionally been used in the aging of capelin (Templeman 1948; Hansen 1943; Pitt 1958; Prokhorov 1965; Winters 1969, 1974). However, the proper interpretation of presumed growth rings on the otoliths of capelin from the western Atlantic has proved difficult and Templeman (1968) called for further validation of the technique. This paper presents the results of such an evaluation.

Materials and Methods

Samples of juvenile and adult capelin were collected with a midwater trawl during the periods May to December 1974 and April to October 1975 (Bailey et al. 1977) in an area extending from Île aux Coudres in the St. Lawrence estuary to Natashquan on the north shore of the Gulf of St. Lawrence and the entrance of Baie des Chaleurs in the southern Gulf (Fig. 1). One subsample of approximately 200 fish was randomly selected from each catch when possible and examined for length frequency and sex composition. A second subsample of approximately 50 fish, also randomly selected, was frozen for later removal of otoliths for aging. Because of the dispersed nature of capelin schools in late summer and fall (Bailey et al. 1977) it was not always possible to obtain the desired number of samples at this time. For this reason the majority of the specimens used in this analysis were collected between June and August.

The degree of shrinkage in length resulting from freezing was determined by measuring and labeling fresh fish, freezing them, and months later thawing and measuring them again. The derived estimate of shrinkage (4%) was used to correct length measurements made on fish frozen prior to measuring.

Total length (TL, in millimetres) was measured from the tip of the mandible with the mouth closed to the tip of the ventral lobe of the caudal fin with this lobe manipulated to point directly backward (Templeman 1948). The otoliths were processed in the laboratory as described by Hourston (1968). Reading of otoliths was done under reflected light using a binocular microscope at 10× magnification with the otoliths immersed in 95% alcohol. Birth date was chosen as January 1 (Templeman 1968). Terminology used is that described by Jensen (1965). Otoliths were read independently by two readers and if disagreement occurred, the otoliths were reread and either accepted after common agreement or rejected. Measurements of the otolith radii were made along a line perpendicular to an axis running from the rostrum to the otolith center (Winters 1974) at a magnification of 40× using an ocular micrometer.

Back-calculated lengths at the time of annulus formation were developed using an empirical curve (Hile 1970) established for the otolith radius–fish length relationship from 589 individuals ranging in total length from 30 to 198 mm (Fig. 2). This curve did not differ for males and females. Winters (1974) reported a difference in similar curves for male and female capelin from the Newfoundland area; however, this difference was significant only for the largest fish in the sample. Length frequencies of capelin larvae from the study area (Jacquaz et al. 1977), converted to total length and corrected for shrinkage due to preservation, were also used in our evaluation of the otolith technique. A series of capelin (10–105 mm TL) collected in the study area and preserved in 10% formalin was examined for changes in internal and external morphology associated with growth.

Results and Discussion

There was an annual periodicity in the formation of hyaline zones on the otoliths of juveniles and adults with the majority being formed in late fall and winter, suggesting that these zones

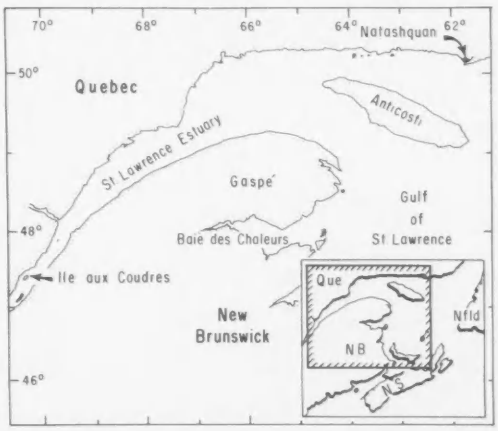

FIG. 1. Study area for capelin in the St. Lawrence estuary and western Gulf. of St. Lawrence.

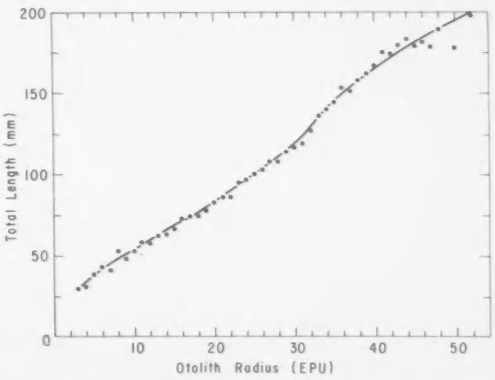

FIG. 2. Relationship between otolith radii and total length, for capelin taken in the St. Lawrence estuary and western Gulf. Ten eye-piece units (EPU) equal 0.2436 mm.

represented true annuli. Resumption of growth in the spring occurred earlier in juveniles than in adults, possibly because of the added stress of spawning experienced by the latter (Fig. 3). Back calculation of lengths at the time of formation of these hyaline zones revealed that the first hyaline zone (Fig. 6), deposited at a mean length of 47 mm (SD 7 mm; Fig. 4), corresponded well with lengths of young-of-the-year capelin collected in plankton tows made within the study area in November 1974 (Fig. 5), when allowance was made for positive bias in the mean length of fish in this sample as a result of gear selection (Jacquaz et al. 1977). This strongly suggests that the first hyaline zone represents the first annulus.

A second hyaline zone (Fig. 6) deposited at a length of 63 mm TL (SD 7 mm; Fig. 4) corresponded precisely with the length at which capelin undergo a profound metamorphosis. Our studies show that prior to the attainment of a length of approximately 50 mm capelin are typically translucent with the exception of two prominent rows of ventral melanophores, lack scales, and have a gut that is straight with no differentiation of the digestive organs. Beginning at 50 mm, stellate melanophores appear on the dorsal and mediolateral surfaces of the posterior portion of the body and by 65 mm TL cover the entire dorsolateral surfaces of the body, the brain, snout, and inner surface of the operculum. In the body cavity, small melanophores develop on the internal lining of the peritoneum, especially dorsal to the gas bladder. At the same length the two prominent ventral rows of large melanophores become less visible externally due to the increasing thickness of the body musculature and the accumulation of iridophores on the exterior surface of the peritoneum.

Differentiation of the digestive organs also occurs at approximately 50 mm, with the stomach and pyloric ceca being first visible as buds at the margin between the esophagus and intestine. At 70–80 mm the interior surface of the peritoneum is completely black, scales form over the posterior portion of the lateral line (personal observation; Pitt 1958), and the gonads are sufficiently differentiated to distinguish between testes and ovaries. By 80 mm individuals resemble adults in internal and external pigmentation, relative arrangement and size of internal organs, and general body proportions.

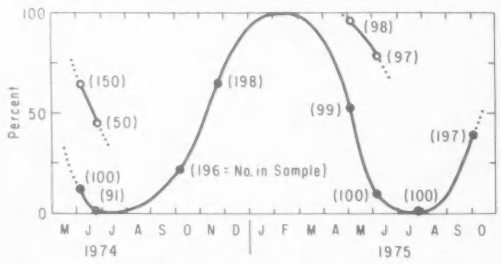

FIG. 3. Relative proportion of capelin otoliths with hyaline edges in the samples collected during 1974 and 1975 from the St. Lawrence estuary and western Gulf. ○ = adults, ● = juveniles.

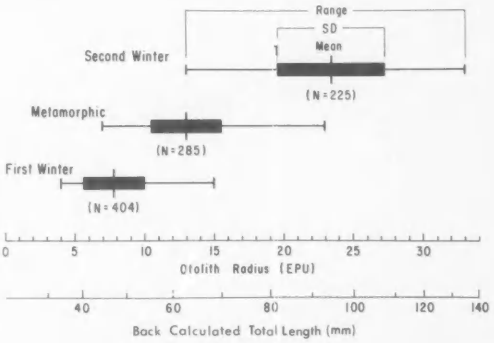

FIG. 4. Back-calculated total length at formation of hyaline zones in juvenile capelin. Ten eye-piece units (EPU) equal 0.2436 mm. ○ = adults, ● = juveniles.

These profound morphological changes, occurring between 50 and 80 mm TL, are believed to result in the formation of the second hyaline zone (metamorphic check) (Fig. 6) laid down at a mean length of 63 mm, which we observed on the otoliths of 77% of the 1- and 2-yr-old fish and 41% of the adults. The development of false annuli under conditions of physiological or physical stress is a well-known phenomenon (Thompson 1926). Our conclusion is supported by an analysis of the otoliths of 21 capelin (50–100 mm TL) which were in the process of their metamorphosis when collected in June 1975. All otoliths had one clearly defined hyaline zone corresponding to the first annulus and 18 (86%) were in the process of developing, or had recently developed (Fig. 6b, c), a second hyaline zone at the border of the otolith.

The absence of a metamorphic check on some otoliths may result from its deposition simultaneously with the first or second annulus. This would occur if individual growth was exceptionally fast, thereby enabling the fish to attain the size of metamorphosis at the end of the 1st yr of growth, or very slow, under which circumstances it would not reach this size until the end of the 2nd yr of growth. The overlap in the range of lengths at which the first and second hyaline zones are deposited and the range of lengths at meta-

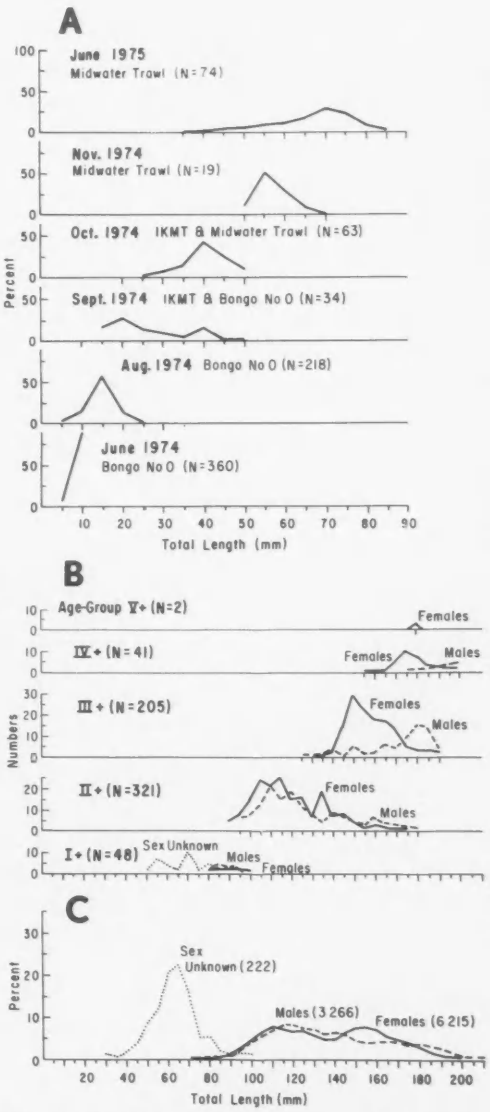

FIG. 5. Length frequencies of larval (June–November 1974) and 1+ (June 1975) capelin from the St. Lawrence estuary and Gulf of St. Lawrence (A); length-frequency distribution of 1975 collection of capelin by age-group (B) and for all samples combined (C).

morphosis (Fig. 4) support this hypothesis. The decreasing frequency of occurrence of metamorphic checks on the otoliths of older fish examined in this study resulted primarily from the obscuring of these checks, which are frequently narrower and less distinct than true annuli, by opaqueness of the otolith which was observed to increase with size and age. In general, the otoliths taken from fish collected in this study were more opaque and thus more difficult to interpret than those from the Newfoundland area (supplied by G. Winters). This suggests that the metamorphic check may be a more prominent and consistent feature on the otolith of capelin from the Newfoundland area. However, differences in 1st-yr growth rates between areas, resulting in different frequencies of overlapping of the metamorphic check with the first or second annulus, could significantly influence the frequency with which the metamorphic check is observed. Of the 39 otoliths of 2-yr-old Newfoundland fish we examined, 92.3% contained metamorphic checks (Fig. 6e, f).

A similar hyaline check has been observed on the otoliths of Atlantic herring (*Clupea harengus harengus*) from the Bay of Fundy. This check, which is deposited at a length of 40–45 mm (Messieh 1975) corresponds closely with the size at metamorphosis in herring (35–40 mm; DeSilva 1974; Graham et al. 1972).

These findings indicate that the peak, occurring between 40 and 85 mm, in the length-frequency histogram of the total catch (Fig. 5) represents fish sampled during the course of their second (1+) growth season. The difference between the mean length at annulus formation (47 mm) and the length-frequency plot of these 1+ fish is attributed to growth that occurred during the 6–10 mo between annulus formation (arbitrarily fixed at January 1) and the time of sampling. This conclusion is supported by the capture of small numbers of larval capelin from various localities around Newfoundland in late May and June 1915–30 (reviewed in Templeman 1948). These larvae, which ranged in length from 42 to 66 cm, were transparent (unmetamorphosed) at time of capture. They were captured before the onset of spawning and were thus clearly a product of the previous year's reproductive effort. Recently (February 27–March 9, 1977) large numbers of unmetamorphosed capelin larvae belonging to the 1976 year-class were captured in the southeastern Newfoundland area. The mean total length of these larvae was 53 mm (range 40–72 mm; Carscadden 1977), indicating that in this area too metamorphosis may occur during the summer of the 2nd yr of life.

A third hyaline zone, deposited at a mean length of 95 mm TL (SD 13 mm; Fig. 4) corresponded with a peak in the length-frequency histogram occurring at approximately 90–130 mm TL (Fig. 5). Again the difference between

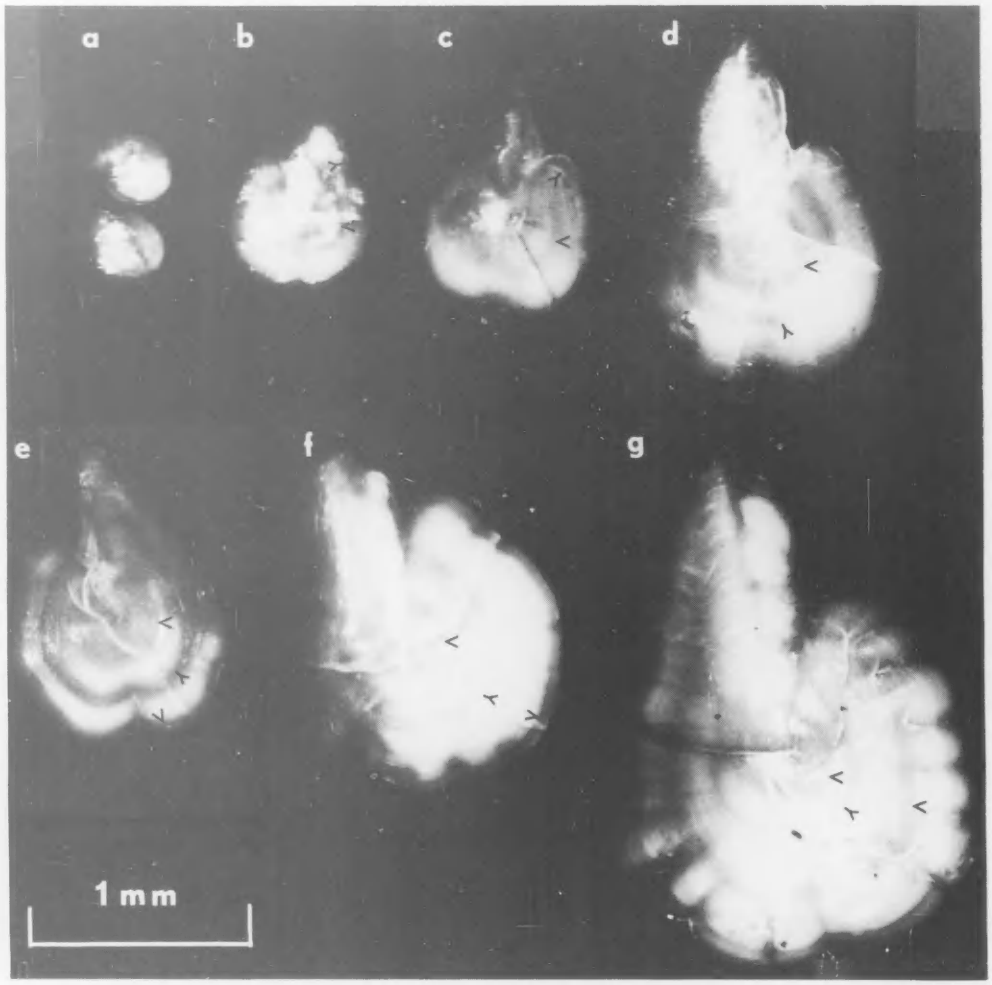

FIG. 6. Capelin otoliths showing annuli (<) and metamorphic checks (≺). a) Age 0+, 47 mm, Pointe des Monts, October 11, 1974; b) Age 1+, 60 mm, Pointe des Monts, June 29, 1974. Metamorphic check forming; c) Age 1+, 77 mm, Pointe des Monts, June 29, 1974. Metamorphic check forming; d) Age 1+, 96 mm, Baie-Comeau, November 28, 1974; e) Age 2, 85 mm. Newfoundland, 49°10′ N, 58°20′ W, May 11, 1974; f) Age 2, 108 mm, Newfoundland, 48°56′ N, 59°11′ W, May 10, 1974; g) Age 2+, 141 mm, Pointe des Monts, October 11, 1974.

length at deposition of the hyaline zone and length at the peak of the length-frequency distribution results from growth during the interval between deposition of the zone and sampling. This hyaline zone deposited at approximately 95 mm TL was thus interpreted as the second annulus.

Length-frequencies of individual age-groups developed using the assumption that all hyaline zones, with the exception of the metamorphic check, were true annuli agreed well with observed peaks in the length-frequency distribution of the total catch when account is taken of growth between the time of annulus formation and sampling (Fig. 5). Similarly, mean back-calculated lengths at age 1 (Table 1) agree closely with the observed lengths (50–60 mm TL) of young-of-the-year capelin sampled in November 1974 when account is taken of the positive bias in the mean length of this sample as a result of gear selection (Jacquaz et al. 1977). There was also general

TABLE 1. Back-calculated total lengths (January 1) of male and female capelin from the estuary and western Gulf of St. Lawrence.

Age-group	No. of fish		Mean length at capture (mm)		Mean calculated total length (mm) at Jan. 1							
					1		2		3		4	
	M	F	M	F	M	F	M	F	M	F	M	F
0	19[a]		46.4[a]		—	—	—	—	—	—	—	—
1	68	116	90.9	86.5	49.4	47.8	—	—	—	—	—	—
2	77	95	121.8	114.8	47.1	45.0	93.3	90.6	—	—	—	—
3	36	42	175.8	156.4	48.4	45.9	106.1	89.9	163.4	141.4	—	—
4	3	4	188.0	175.8	50.3	49.5	89.7	95.5	153.3	146.3	179.0	169.0
			Weighted mean length		48.3	46.5	97.2	90.5	162.6	141.8	179.0	169.0
				SE	5.7	6.4	14.5	14.1	15.7	14.4	10.0	13.1

[a]Sex unknown.

agreement in back-calculated estimates of mean length at age developed from fish of different ages, and between the weighted mean length at annulus formation and the mean length of equivalent ages at capture (after allowance for growth).

Templeman (1968) compared the back-calculated lengths of capelin at the time of formation of each hyaline zone as determined by the "Greenland" or "Danish" method and the "Newfoundland" method. Because of the absence of direct information on the lengths of capelin at the end of the first growing season he was unable to determine which of the two methods was correct and therefore called for a further evaluation of the techniques. Our results lead us to conclude that the Greenland method, which correctly interprets the first annulus but apparently considers the metamorphic check to be the second winter zone, when applied to capelin from the estuary and western Gulf of St. Lawrence, results in ages that are 1 yr too old. The Newfoundland method considers the first annulus to be a "larval check ring" (a term having no biological basis but used by Templeman (1948, 1968) for purposes of discussion) and apparently interprets the metamorphic check as the first annulus, thus giving correct age estimates, but lengths at the first annulus are too great. This generalization appears to hold for capelin from the Newfoundland region as well. For example, Templeman (1968) reported a range of 28–44 mm TL for Newfoundland capelin at the time of deposition of the first hyaline zone (which he called "larval check ring"). This is in good agreement with the length at formation of the first annulus as determined in this study (Fig. 4), considering that capelin spawn 1–2 mo earlier in the estuary and western Gulf, and therefore have a longer growing season in their 1st yr. The reported back-calculated average lengths at the time of formation of the second hyaline zone (interpreted by Templeman to be the first annulus) was 66–71 mm, which corresponds almost exactly with our determination of the length at metamorphosis.

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Seasonal Distribution, Abundance, and Growth of Larval Capelin (*Mallotus villosus*) in the St. Lawrence Estuary and Northwestern Gulf of St. Lawrence[1]

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Capelin (*Mallotus villosus*) larvae in the St. Lawrence estuary and northwestern Gulf of St. Lawrence were most abundant in 1974 and 1975 in the upper estuary in early June; this coincided with the spring peak in primary production. A spectacular decline (>99%) in larval abundance between June and September resulted principally from their downstream transport by currents. In October and November larval capelin were concentrated in a large counterclockwise gyre located in the northwestern Gulf between Anticosti Island and Pointe des Monts. This region is within the area of maximum primary production in the estuary and Gulf and may serve as an important feeding area for larval capelin. Length of capelin larvae at the end of the first growing season in the estuary and northwestern Gulf was greater than that of capelin from Newfoundland, Labrador, and Greenland. However, this difference may have resulted from sampling biases. Daily growth rates of capelin larvae from these areas were similar.

Key words: capelin, *Mallotus villosus*, St. Lawrence, seasonal distributions, larval transport, growth, vertical distribution

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Le maximum d'abondance de larves de capelan (*Mallotus villosus*) dans l'estuaire et le nord-ouest du golfe Saint-Laurent se trouva en 1974 et 1975 dans le haut estuaire au début de juin; ceci coïncida avec le pic printannier de production primaire. Le transport en aval par les courants a été le principal facteur d'un déclin spectaculaire (>99%) de l'abondance des larves entre juin et septembre. En octobre et novembre, les larves de capelan étaient concentrées dans un grand tourbillon océanique en sens inverse des aiguilles d'une montre situé dans la partie nord-ouest du Golfe, entre l'île d'Anticosti et Pointe des Monts. Ce secteur est situé dans la région de production primaire maximale de l'Estuaire et du Golfe, et peut être une aire d'alimentation importante pour les larves de capelan. A la fin de la première saison de croissance, les larves de capelan de l'Estuaire et du nord-ouest du Golfe sont plus grandes que celles de Terre-Neuve, du Labrador et du Grœnland. Cependant, il est possible que cette différence soit le résultat d'erreurs systématiques d'échantillonnage. Les rythmes de croissance quotidienne des larves de capelan provenant de ces régions sont identiques.

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CAPELIN (*Mallotus villosus*) are an important component of the marine ecosystem in boreal-arctic waters of the North Atlantic where they comprise a significant portion of the diet of many fishes, marine mammals and birds (Bailey et al. 1977b).

In recent years commercial exploitation of capelin has increased dramatically (Suda 1973; Jangaard 1974; Winters 1975; Carscadden 1976). Rational utilization of this resource requires a better understanding of capelin biology than presently exists. Knowledge of the early life history of capelin may be of particular importance in understanding the factors affecting survival and

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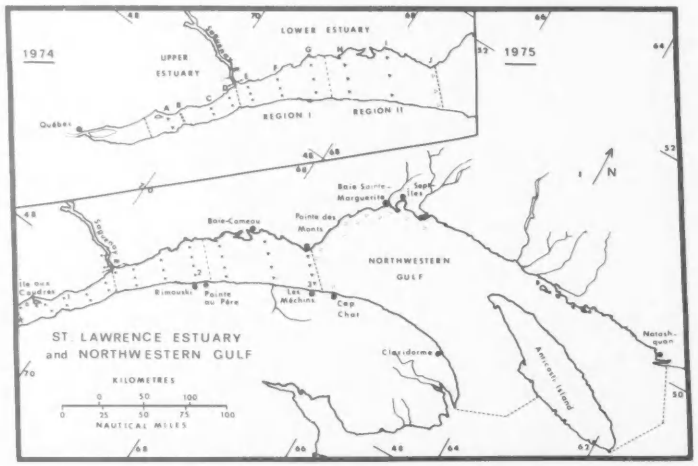

FIG. 1. Sampling locations and periods for capelin larvae in the St. Lawrence estuary and northwestern Gulf of St. Lawrence during 1974 and 1975. Symbols indicate periods of sampling at stations, 1974: June–October (●), July–October (○), late July–October (▼), late August–October (▽); 1975: late May–early June (○), late May–September except end of June (●), end of June (▽), and May–September (▼). Numbered stations (1975) indicate 24-h sampling locations: June 3–4 (1), August 4–5 (2), and September 15–16 (3). In 1975 transect A includes all the stations around Île aux Coudres.

subsequent year-class strength, a concept originally proposed by Hjort (1914) and recently discussed by May (1974) and Bannister et al. (1974).

Knowledge of the ecology and distribution of larval capelin in Canadian waters is sparse being limited to a description of the larvae around Newfoundland (Templeman 1948), length and distribution of the larvae from the southern Gulf of St. Lawrence and southern Newfoundland (Winters 1969, 1974; Hodder and Winters 1972), and records of the occurrence of larvae in Baie des Chaleurs and the southwestern Gulf of St. Lawrence (Bergeron and Lacroix 1963; Lacroix and Bergeron 1964) and the southern Gulf (Kohler et al. 1974a, b, 1975).

This paper discusses the seasonal distribution and abundance of capelin larvae in the estuary and northwestern Gulf of St. Lawrence and relates these to relevant physical and biological patterns.

Materials and Methods

GEAR TYPES

In 1974 a ½-m bongo sampler equipped with closing devices (McGowan and Brown 1966), flowmeter, and time-depth recorder (TDR), and fitted with No. 0 (0.51-mm) mesh nets was used to collect capelin larvae at all fixed stations. In 1975 the same gear was used but it was equipped with one No. 0 (0.51-mm) and one No. 10 (0.15-mm) mesh net except in September when only collections from the No. 0 mesh net were used because the No. 10 nets tore at the higher tow speeds used.

Additional miscellaneous information on the distribution of larvae in the estuary, the northwestern Gulf, and the Saguenay River was obtained from samples taken at irregular intervals by an Isaaks Kidd midwater trawl (IKMT) fitted with a 1.3-cm nylon liner and a No. 3 (0.32-cm) nylon cod end, a Boris midwater trawl (Bailey et al. 1977b), a 57-cm No. 0 mesh plankton net, and the bongo sampler fitted with No. 0 mesh net. The Boris trawl catches were small because of the large mesh size of the gear. The length frequencies of catches obtained with the Boris and IKMT are also believed to have been positively biased as a result of their larger mesh sizes.

Salinity, temperature, and depth data were obtained with a STD recorder or bathythermograph and Nansen bottle casts were made at each station during each sampling period.

SAMPLING TECHNIQUES

Sampling for capelin larvae was conducted at a number of fixed stations in the St. Lawrence estuary between Île aux Coudres and Pointe des Monts, as well as at several stations in and around the mouth of the Saguenay River and the western Gulf of St. Lawrence (Fig. 1). In 1974, samples were collected

at approximately 3-wk intervals between June and October at 28 stations (227 tows), arranged in 10 transects. In 1975, 37 stations (375 tows), along essentially the same transects, were sampled at approximately 5-wk intervals from June to September. A series of 11 stations between the Gaspé Peninsula and Sept-Îles were sampled during June 25–29, 1975, and additional sampling was conducted in the lower estuary and western Gulf at irregular intervals. Three stations were sampled at 4-h intervals over a 24-h period in June, August, and September 1975 to determine the vertical distribution of larvae and to explore the extent of net avoidance by larger larvae.

At each station three 10-min step-oblique tows were made. The sampling speed was 2–3 knots except in September 1975 when we sampled at 4–5 knots to reduce net avoidance by the larger larvae. In 1975 the depths of the tows varied with the season in response to seasonal differences in the vertical distribution of the larvae observed during 1974. From May 31 to June 16, 1975 we attempted to sample 0–5, 6–25, and 26–50 m; from July 30 to August 9 the sampling depths approximated 0–25, 26–50, and 51–100 m. In September 1975 we attempted to sample 0–50, 51–100, and 101–150 m. The average of the actual depths sampled (based on TDR readings) approximated the intended sampling depths (Table 6).

The approximate sampling depths of the 24-h stations were 0–5, 6–25, and 26–50 m (June 3–4 and August 4–5) and 0–50, 51–100, and 101–150 m (September 15–16).

The IKMT collections obtained in September 1974 were derived from 15-min tows at each station at an average depth of 30 m. The October samples came from 3 15-min step-oblique tows at 0–50, 50–100, and 100–150 m (where depth permitted) at each station.

Treatment of Samples and Data

All samples were preserved in 5% buffered formalin immediately after capture. In 1974, only the samples collected in the left net of the bongo sampler were sorted. In 1975, when two mesh sizes were used, the samples from both nets were sorted. Large samples were subsampled with a Folsom plankton splitter. Several collections containing large numbers of larvae were subsampled 3 times and the mean was used for the estimate of abundance (Horwood and Driver 1976). Capelin larvae were separated from larval rainbow smelt (*Osmerus mordax*) and Atlantic herring (*Clupea harengus harengus*) using criteria described by Templeman (1948).

In this paper the term larvae is used to describe all capelin spawned in the year of sampling. To insure that this criteria was met the otoliths of large larvae collected in the fall samples were read to determine their age (Bailey et al. 1977a) and larvae spawned the year prior to sampling were excluded.

In 1974 all capelin larvae and in 1975 yolk-sac and capelin larvae were counted and their abundance was expressed as number of larvae per cubic metre or per 100 cubic metres of water filtered. Standard length (SL) was determined for 50–60 larvae selected at random from each sample in 1974 and 20–25 larvae selected in 1975. Larvae <20 mm were measured with an ocular micrometer to the nearest 0.1 mm, while larger larvae were measured to the nearest millimetre with a ruler in 1974 and to the nearest 0.1 mm with a dial caliper in 1975.

The filtering efficiency of the bongo sampler was calibrated by comparing the bongo flowmeter readings with those of a precalibrated TSK (Tsumuri-Seiki Kosakusho Co., Ltd.) flowmeter mounted in the center of the opening of the bongo frame. In addition, during June–August 1975 a flowmeter was generally mounted in the frame of the No. 0 mesh net. The volume filtered through the No. 0 mesh net was used in calculations of larval abundance in the No. 10 net. Had clogging occurred in the No. 10 net a negative bias in estimates of the volume filtered would occur. Net clogging did not appear to be a problem and its influence on larval abundance was disregarded in the analysis.

Variances in mean number of capelin larvae per unit volume of water filtered were generally greater than the mean indicating nonnormally aggregated distributions (Chanut 1975). A logarithmic transformation was therefore performed to normalize the data (Barnes 1952; Cassie 1971). For comparative purposes we separated the estuary into three regions (Fig. 1) and analyzed the data from these regions, the Saguenay River, and the northwestern Gulf as separate units. The western boundary of the sampling area corresponds to the approximate upper limit of the saltwater wedge (Greisman and Ingram, McGill University, Marine Science Centre, personal communication). The boundary between the upper estuary and the lower estuary is based on differences in the bottom physiography (d'Anglejan and Smith 1973). The lower estuary has been arbitrarily divided into two regions (I and II) (Fig. 1).

The significance of temporal and spatial differences in mean length and mean abundance of capelin larvae was tested by single classification analysis of variances (Dixon and Massey 1969) and Student's *t*-test. Differences between the proportion of yolk-sac larvae in different regions were examined by chi-square tests.

The zooplankton from the No. 0 mesh samples (upper 50 m) were dried at 60°C for 24 h, weighed to the nearest 10 mg, and expressed as milligrams per cubic metre.

Results

Gear Efficiency

The No. 10 (0.15-mm) mesh net was much more efficient than the No. 0 (0.51-mm) in collecting capelin larvae during simultaneous sampling with the bongo sampler in 1975. This was especially true early in the sampling period (May–June) when the larvae were small (Table 1). However, the No. 0 mesh net caught larger larvae during the last two sampling periods

(Table 1). The relation between the catchability of the two nets at selected stations during June 1975 is described by the equation

$$y = x^{1.5741} \ (r = 0.847)$$

where

$y = 1 +$ (the abundance of capelin larvae per 100 m^3 of water filtered through the No. 10 mesh net)

$x = 1 +$ (the abundance of capelin larvae per 100 m^3 of water filtered through the No. 0 mesh net)

This equation was used to compare the relative abundance of capelin larvae captured in June 1974 and 1975.

Dominance

Capelin larvae dominated the ichthyoplankton collected in the entire sampling area during 1974 and 1975 (Table 2). In 1975 86% of the 617,525 larvae collected was capelin, with a slight seasonal decline from 92% of the total larvae in June to 58% in September; 82% of the larval samples collected in the northwestern Gulf during the latter part of June was capelin. The frequency of occurrence of capelin larvae at the stations sampled in 1975 (97% in June to 33% in September) showed similar trends. In 1974 55% of the 5893 larvae collected was capelin, but after June the relative abundance and frequency of occurrence of capelin appears to be less than in 1975.

The upper limit of sampling in 1975 (Fig. 1) appears to correspond to the upper limit of significant larval capelin occurrence in the estuary. At the westernmost three stations above Île aux Coudres, capelin made up only 14% of the total larvae in June, the remainder being primarily *O. mordax*.

TABLE 1. Differences in abundance (geometric means of larvae per 100 m^3 filtered) and standard length (mean and standard deviation in millimetres) of capelin larvae collected simultaneously with No. 10 (0.15-mm) and No. 0 (0.51-mm) mesh nets in 1975.

Sampling period	Mesh Net		Significance of differences
	No. 10	No. 0	
May 31–June 16			
Abundance	60.9	9.1	$P<0.01$
Length	5.9 (1.05)	5.5 (0.80)	$P<0.01$
June 25–June 29			
Abundance	4.1	1.7	$P<0.01$
Length	5.8 (0.93)	6.2 (1.81)	$P<0.01$
July 30–Aug. 9			
Abundance	2.3	1.2	$P<0.01$
Length	5.8 (1.88)	10.6 (4.09)	$P<0.01$

Hatching Periodicity

Yolk-sac larvae were collected during every cruise between May 31 and September 24, 1975 and during the course of the year from virtually every station sampled (Fig. 2E, F, G, Table 3).

These larvae were most abundant in the upper estuary and region I of the lower estuary in May and early June, and in the northwestern Gulf in late June (Fig. 2E, F; Table 3). A second smaller peak occurred in the upper estuary and region I of the lower estuary in late July–early August (Fig. 2G; Table 3).

The decline in abundance of yolk-sac larvae between June and September (Table 3) is probably underestimated because of the use of the larger No. 0 net, which is less efficient for smaller larvae (Table 1). In early June the largest relative number of yolk-sac larvae occurred along the south shore of region I and the north shore of region II in the lower estuary and the lowest relative number occurred on the south shore of region II (Fig. 2E). From June 25 to 29 yolk-sac larvae continued to be most abundant on the north shore of the lower estuary (region II) and in Baie Sainte-Marguerite and Sept-Îles area of the northwestern Gulf (Fig. 2F). The relative abundance of yolk-sac larvae in the upper estuary and region I of the lower estuary during May 31–June 16, 1975 was comparable to the relative abundance of yolk-sac larvae in the northwestern Gulf near Baie Sainte-Marguerite during June 25–29. The relative abundance of yolk-sac larvae in region II of the lower estuary was significantly less ($P \leqslant 0.01$) than in the above-mentioned areas (Table 3). Relative abundances of yolk-sac larvae in the lower estuary were significantly lower ($P \leqslant 0.01$) during July 30–August 9 than in earlier sampling periods (Fig. 2F). At this time a high yolk-sac larvae concentration occurred at the mouth of the Saguenay River (Fig. 2F). In the area north of Anticosti Island the percentage of yolk-sac larvae at five stations located approximately midway between the island and the north shore (Fig. 5) which were sampled during July 31–August 4, 1975 ranged from 0 to 57%. During September 14–24 three yolk-sac larvae were collected (two at transect H, south shore; one at transect G, south shore). Yolk-sac larvae occurred only in small numbers in the Saguenay River (Table 3).

Developing capelin eggs were collected in the water column in the upper estuary at depths of 5–20 m during May 31–June 2, 1975 (1.3–31.7

TABLE 2. Number (*N*), relative abundance (percent of total larvae) (A), and frequency of occurrence (percent of tows with capelin larvae) (B) of capelin larvae in the sampling areas. Data from collections taken with a No. 0 (0.51-mm) mesh net in 1974 and No. 10 (0.15-mm) mesh net (May–August) and No. 0 net (September) during 1975.

Sampling period	Upper estuary			Lower estuary Region I			Lower estuary Region II			Northwestern Gulf of St. Lawrence			Saguenay River			All areas combined		
	N	A	B	*N*	A	B	*N*	A	B	*N*	A	B	*N*	A	B	*N*	A	B
1974																		
June 7–12	2,371	98.1	85.7	175	98.9	75.0	—	—	—	—	—	—	—	—	—	2,546	98.0	80.0
July 2–6	122	43.8	100.0	197	77.9	66.7	—	—	—	—	—	—	1	33.3	100.0	320	68.0	82.4
July 29–Aug. 3	4	6.3	33.3	71 x	74.0	88.9	240	99.6	83.3	—	—	—	15	65.2	100.0	330	77.3	68.0
Aug. 31–Sept. 9	0	0.0	0.0	2	20.0	11.1	24	82.8	33.3	—	—	—	1	50.0	100.0	27	38.0	17.9
1975																		
May 31–June 16	407,052	89.4	100.0	95,575	99.9	90.9	19,040	99.6	100.0	—	—	—	2,028	99.6	100.0	523,695	91.6	97.3
June 25–29	—	—	—	—	—	—	1,766	99.1	100.0	1,024	82.2	54.6	—	—	—	2,790	92.1	73.7
July 30–Aug. 9	35	24.1	30.0	2,178	95.7	90.9	84	63.6	58.3	—	—	—	92	86.8	50.0	2,389	89.9	60.0
Sept. 14–24	4	12.1	36.4	0	0.0	0.0	47	92.2	66.7	—	—	—	0	0.0	0.0	51	58.6	33.3

TABLE 3. Total number of capelin larvae (*N*), percent yolk sac (A), and percent frequency of occurrence of yolk-sac larvae (B) in positive tows with No. 10 (0.15-mm) mesh net (May–August) and No. 0 (0.51-mm) net (September), 1975.

Sampling period	Upper estuary			Lower estuary Region I			Lower estuary Region II			Northwestern Gulf of St. Lawrence			Saguenay River			All areas combined		
	N	A	B	*N*	A	B	*N*	A	B	*N*	A	B	*N*	A	B	*N*	A	B
May 31–June 16	407,052	40.3	100.0	95,575	58.1	80.0	19,040	26.1	91.7	—	—	—	2,028	3.7	100.0	523,695	42.6	91.7
June 25–29	—	—	—	—	—	—	1,766	21.7	75.0	1,024	42.6	83.3	—	—	—	2,790	33.9	78.6
July 30–Aug. 9	35	71.4	100.0	2,178	14.2	71.4	84	4.8	28.6	—	—	—	92	13.0	100.0	2,389	15.1	61.1
Sept. 14–24	4	0.0	0.0	0	—	0.0	47	4.3	12.5	—	—	—	0	—	0.0	51	4.1	8.3

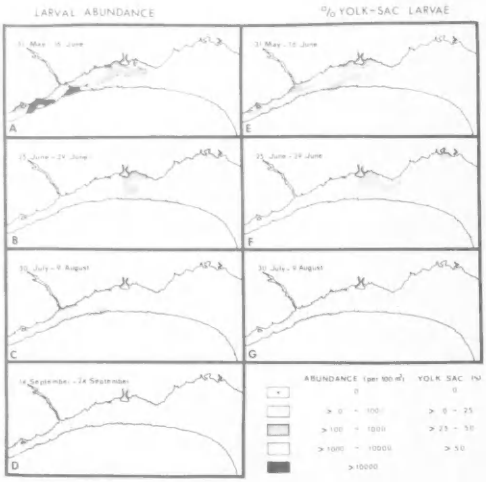

FIG. 2. Seasonal distribution and abundance of total capelin larvae (A–D) and yolk-sac larvae (E–G) in the St. Lawrence estuary and northwestern Gulf, 1975, based on bongo sampler collections with a No. 10 (0.15-mm) mesh net in May–August (0–32 m) and a No. 0 (0.51-mm) mesh net in September (0–50 m).

eggs/100 m³) and at the mouth of the Saguenay River at 0–32 m during August 7–9, 1975 (1.5–23.9 eggs/100 m³). Saetre and Gjøsaeter (1975) have also reported the pelagic occurrence of developing capelin eggs.

DISTRIBUTION AND ABUNDANCE

During 1974 and 1975 capelin larvae occurred throughout the estuary, the lower Saguenay River, and the northwestern Gulf, but patterns of distribution and centers of abundance showed marked seasonal changes in both years. In June capelin larvae were most abundant in the upper estuary and along the south shore of the lower estuary (region I) (Fig. 2A, 3A). During June 25–29, 1975 restricted concentrations occurred along the north shore of the northwestern Gulf (Fig. 2B). By late July–early August of both years the areas of maximum abundance had shifted to the lower estuary but the distribution of larvae within this area varied between 1974 and 1975 (Fig. 2C, 3C). In the fall of both years capelin larvae were most abundant in the lower estuary (Fig. 2D, 3D, Table 4). During August 31–September 9, 1974 capelin larvae occurred only in bongo collections from the lower estuary (transects I, J, Fig. 3D) but IKMT collections obtained at the same time revealed the presence of larvae in the upper estuary as well (Fig. 4A).

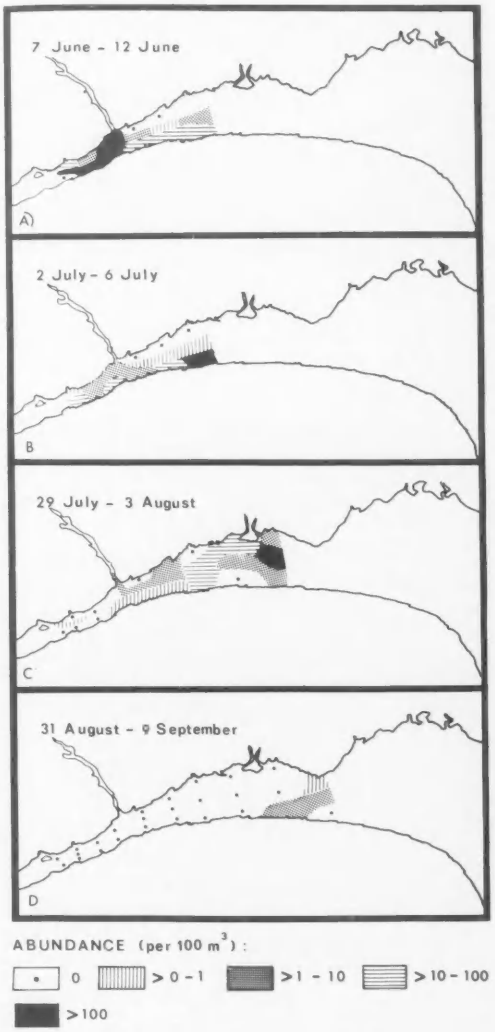

FIG. 3. Seasonal distribution and abundance of capelin larvae in the St. Lawrence estuary, 1974, based on bongo sampler collections with a No. 0 (0.51-mm) mesh net in the upper 50 m of the water column.

A similar distribution occurred in September 1975, although no larvae were collected in region I of the lower estuary and larvae were collected further to the west in the upper estuary (Fig. 2D).

Capelin larvae were also collected north and west of Anticosti Island during July 31–August 4, 1975, and October 1–18, 1975 (Fig. 4C). In October 1974 and 1975 larval capelin were collected at the majority of the stations sampled in

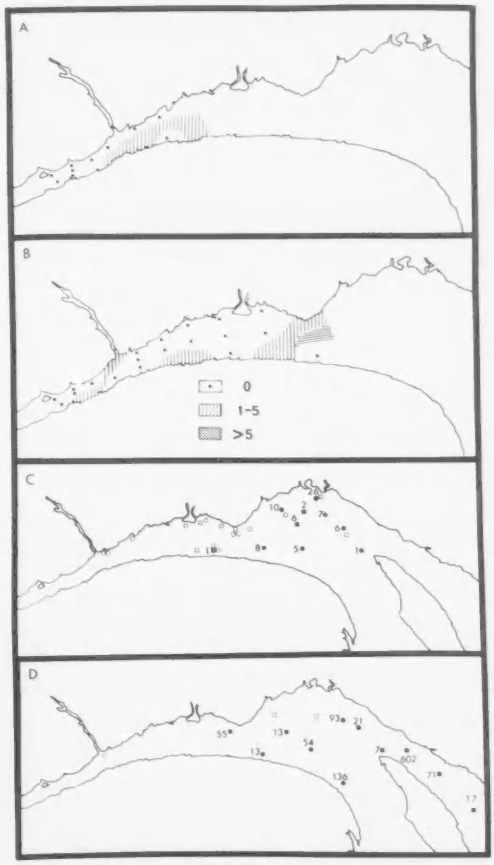

FIG. 4. Distribution and abundance (larvae per station) of capelin larvae in the St. Lawrence estuary September 4–9 (A) and October 3–17, 1974 (B), based on IKMT collections; numbers of capelin larvae in midwater trawl collections from the estuary and northwestern Gulf October 12–16 (●) and November 23–25 (■) 1974, (C); and numbers of capelin larvae collected at miscellaneous stations in the lower St. Lawrence estuary and northwestern Gulf, 1975 (D) (■) 57 cm plankton net, July 31–August 4. (●) midwater trawl, October 1–12. Open symbols indicate no larvae in sample.

the northwestern Gulf but at only one station in the lower estuary (Fig. 4C, D). Results of midwater trawl collections during November 1974 suggest that at this time capelin larvae are largely restricted to the northwestern Gulf (Fig. 4D). The small number of larvae collected resulted primarily from the large mesh of the gear and is not a reflection of numbers available. In October 1974 and 1975, and November 1974 larval, juvenile, and adult capelin occurred to-

TABLE 4. Abundance (larvae per 100 m³ filtered) (A) and standard length (number measured, mean, and standard deviation in millimetres) (B) of capelin larvae collected in five sampling areas with No. 0 (0.51-mm) mesh net, 1974, and No. 10 (0.15-mm) (May–August) and No. 0 mesh net (September), 1975.

Sampling period	Upper estuary		Lower estuary						Northwestern Gulf of St. Lawrence		Saguenay River		All areas combined	
			Region I		Region II									
	A	B	A	B	A	B			A	B	A	B	A	B
1974														
June 7–12	194.7	305 5.8(0.55)	9.6	115 5.3(0.45)	—	—			—	—	—		87.3	420 6.5(0.57)
July 2–6	7.3	116 5.1(0.48)	7.4	78 5.3(0.40)	—	—			—	—	0.2	1 6.3	6.9	195 5.2(0.46)
July 29–Aug. 3	0.1	4 8.2(4.65)	3.8	69 11.4(3.81)	8.7	135 12.4(2.20)			—	—	2.7	18 15.26(3.48)	4.7	226 12.2(3.10)
Aug. 31–Sept. 9	0.0		0.0	2 28.0(2.83)	5.8	24 17.1(4.33)			—	—	0.2	1 30.0	0.2	27 18.4(5.61)
1975														
May 31–June 16	7130.4	638 5.5(0.62)	1451.6	426 5.5(0.68)	263.7	493 6.1(1.15)			—		161.6	114 7.8(0.98)	2521.9	1671 5.9(1.05)
June 25–29	—	—	—	—	33.1	303 5.9(0.83)			18.0	146 5.7(1.68)	—	—	25.3	449 5.8(0.93)
July 30–Aug. 9	0.8	35 4.9(0.27)	36.9	302 5.1(0.43)	1.3	82 8.6(2.91)			—	—	7.4	25 5.4(0.40)	13.3	444 5.8(1.88)
Sept. 14–24	0.1	3 27.2(9.76)	0.0	—	0.4	37 25.9(7.92)			—	—	0.0	—	0.2	40 26.0(7.93)

TABLE 5. Ranges of temperature and salinity at approximate depths of capture of capelin larvae and total range of temperature and salinity observed at sampling sites, 1975.

Sampling period	Depths of recorded temp and salinity (m)	Temp range (°C)	Temp range (°C) at which capelin were collected	Salinity range (‰)	Salinity range (‰) at which capelin were collected
May 31–June 16	0 & 15	2.4–12.8	2.4–12.8	4.8–29.0	4.8–29.0
June 25–29	0 & 15	2.0–12.6	2.0–12.6	21.5–32.2	21.5–31.5
July 30–Aug. 9	0 & 15	1.6–16.8	1.6–14.4	10.6–32.0	15.5–32.0
Sept. 14–24	15 & 25	0.0–7.0	0.2–7.0	23.6–33.1	26.0–32.6
All observations combined		0.0–16.8	0.2–14.4	4.8–33.1	4.8–32.6

gether in midwater trawl collections taken from the northwestern Gulf (Fig. 4C, D; Bailey et al. 1977b).

Capelin larvae were collected in the lower Saguenay River during most sampling periods in 1974 and 1975 (Tables 2, 4) and were collected as far as 43 km up the Saguenay in September 1975.

Capelin larvae occurred over almost the entire range of temperatures and salinities measured in the upper portion of the water column in the estuary during June–September 1975. The occurrence of these larvae at cooler temperatures in September reflected seasonal cooling of the water near the surface as well as the occurrence of larvae deeper in the water column and below the thermocline (Table 5).

The decline in larval abundance in the estuary between June and September of 1974 and 1975 was spectacular (Fig. 5, Table 4). In June 1975 capelin abundances averaged 26.51 larvae/m^3 (maximum 1115.4/m^3) and 98.9% of all capelin larvae collected in 1975 were taken in that month. Of these, 77% were collected in the upper estuary. In September 1975, when 0.01% of all capelin larvae were collected, abundances in the same area averaged only 0.0025 larvae/m^3 (maximum 0.03/m^3).

No significant difference ($P > 0.05$) was observed in the relative abundance of capelin larvae collected in No. 0 mesh nets fished in the estuary during June 1974 and 1975. Similarly no significant ($P > 0.05$) difference in relative abundance occurred when corrected No. 0 mesh catches (June 1974) and No. 10 mesh catches (June 1975) were compared. The relative abundance of larvae was greater in August 1974 ($P < 0.01$) but not significantly different in September ($P > 0.05$).

VERTICAL DISTRIBUTION

During June–August 1974 and 1975 over 98% of all capelin larvae were collected in the upper

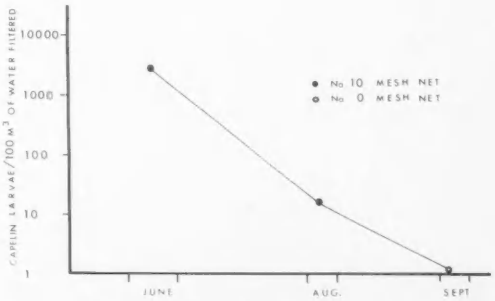

FIG. 5. Relative abundance of capelin larvae in the St. Lawrence estuary during the sampling periods, 1975.

50 m of the water column (Table 6). In June 1974 and 1975, over 99% of all larvae were collected between 0 and 34 and 0 and 26 m, respectively. In August 1974, 98% of all larvae occurred between 0 and 50 m and in August 1975, 89% of all larvae occurred between 0 and 32 m. In September of 1974 and 1975 the proportion of larvae at depths >50 m increased to 17 and 28%, respectively.

Yolk-sac larvae were most abundant in the upper 25 m of the water column. During May 31–June 16 an exception to this pattern occurred when the greatest proportion of the yolk-sac larvae collected in the upper estuary occurred at 21–43 m in the water column.

In the 1974 samples collected August 31–September 9 there was a slight tendency toward an increase in mean larval length with increasing depth; however, this pattern was not repeated in 1975.

No evidence of vertical migration by capelin larvae (3.9–40.2 mm SL) was seen in day–night comparisons conducted at specific stations during June and August 1975 (Table 7). In September 1975 sample sizes were too small to provide a clear indication of behavior. Day–night samples provided no evidence of net avoidance by capelin larvae during daylight sampling with the possible

TABLE 6. Vertical distribution and abundance (individuals/100 m^3 filtered) of total larvae and yolk-sac larvae and mean lengths of larvae at various depths in the St. Lawrence estuary and Saguenay River, 1974 and 1975. (Data from No. 0 (0.51-mm) net collections in 1974 and September 1975 and No. 10 (0.15-mm) net collections in May–August 1975.)

Sampling period	Sampling depth (m)	% total larvae	% yolk-sac larvae	Abundance	N	Length (mm) $\bar{x}$ (SD)
1974						
June 7–12	0–34	99.9	—[a]	94.5	417	5.6 (0.56)
	53–84	0.1	—	2.3	3	4.8 (0.40)
	69–111	0.0	—	0.0		—
July 2–6	0–43	99.7	—	13.8	194	5.2 (0.45)
	51–99	0.0	—	0.0		—
	82–152	0.3	—	1.2	1	5.9
July 29–Aug. 3	0–50	98.2	—	9.1	213	12.2 (3.07)
	54–110	1.5	—	0.3	9	14.6 (3.95)
	92–166	0.3	—	0.9	4	6.9
Aug. 31–Sept. 9	0–38	83.0	—	0.3	18	15.7 (3.19)
	40–79	13.7	—	0.2	7	22.3 (4.35)
	67–119	3.3	—	0.1	2	30.0
1975						
May 31–June 16	0–4	27.7	22.9	1939.5	773	5.9 (1.00)
	5–20	72.0	76.9	5227.5	736	5.9 (1.08)
	21–43	0.3	0.2	30.4	162	5.3 (0.85)
June 25–29	0–5	60.2	37.3	48.1	260	5.9 (1.15)
	7–26	37.7	60.7	29.0	177	6.0 (1.21)
	24–62	2.1	2.0	1.5	12	4.5 (0.72)
July 30–Aug. 9	0–32	89.6	54.3	25.7	202	5.6 (1.47)
	24–62	10.4	45.7	4.2	100	7.2 (4.17)
	50–111	0.0	0.0	0.0		—
Sept. 14–24	0–47	72.0	100.0	0.4	26	24.4 (9.73)
	45–88	14.0	0.0	0.1	7	29.0 (5.58)
	75–124	14.0	0.0	0.2	7	27.4 (2.15)

[a]No data.

exception of the August 4–5 sample at the deepest depths (Table 7).

ZOOPLANKTON DISTRIBUTION AND BIOMASS

In 1975, zooplankton biomass was highest in the upper estuary during most sampling periods and in the northwestern Gulf during June 25–29 (Fig. 6). However, during July 30–August 9 zooplankton biomass was greatest in the lower estuary (region II) though not significantly so ($P \geq 0.05$). Sampling over a 24-h period in June, August, and September 1975 indicated zooplankton biomass was greater in the upper portion of the water column at night but significantly so only in August ($P \leq 0.01$). There was no correlation between larval and zooplankton abundance during any sampling period.

GROWTH

Growth rates of capelin larvae in the St. Lawrence estuary during 1974 and 1975 were similar (Fig. 7). Monthly estimates of daily growth rate during July–October 1974 and 1975 ranged between 0.2 and 0.35 mm. In November 1974 capelin larvae collected with a midwater trawl in the northwestern Gulf averaged 48.4 mm. The mean length of the November sample is believed to have been positively biased by gear selection, a conclusion supported by the apparent, but biologically unreasonable, acceleration in growth in the October–November interval (0.37 mm/day) relative to the September–October period (0.11 mm/day). With allowance for additional growth between November and June, the average size of these larvae conforms well with the size of 1-yr-old capelin collected in June 1975 (Fig. 8) and we conclude that the two samples represent the same year-class.

Differences in the average length of larvae collected at the same time from different areas in the estuary are apparent at every sampling period with the largest larvae usually found in the lower estuary and the northwestern Gulf (Fig. 9).

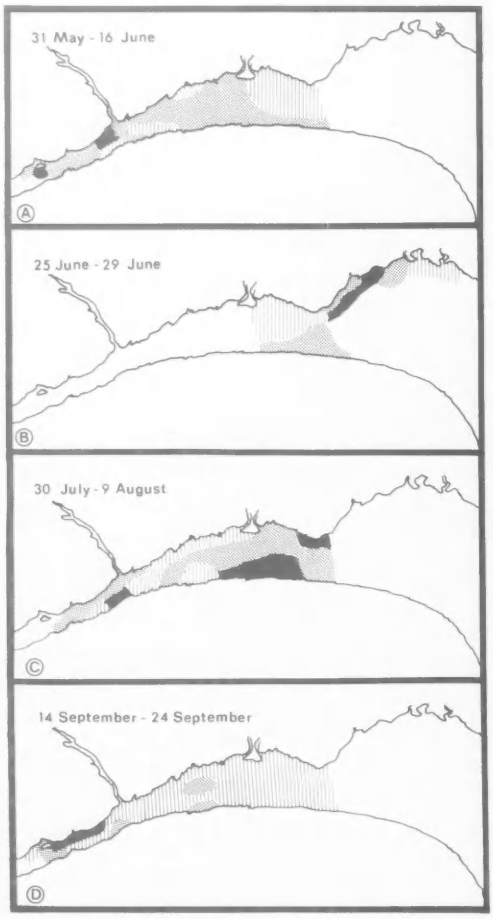

FIG. 6. Seasonal distribution and relative biomass of zooplankton in the St. Lawrence estuary and northwestern Gulf, 1975, based on collections with a No. 0 (0.51-mm) mesh net in the upper 50 m of the water column.

Discussion

Spawning Times

Parent and Brunel (1977), in their extensive review of historical and recent capelin spawning areas and dates in the estuary and Gulf, report beach spawning begins in the upper estuary in late April and occurs progressively later at beaches in the lower estuary and northwestern Gulf, where spawning typically occurs between mid May and mid July.

The timing of the occurrence of yolk-sac larvae in the estuary and northwestern Gulf generally agreed with reported spawning times for different localities (Parent and Brunel 1977) when allowance was made for development and hatching time (15 days; Jeffers 1931; Templeman 1948). However, the occurrence of yolk-sac larvae in the estuary during July 30–August 9 and on September 14 and 20, 1975 cannot be accounted for on the basis of reported spawning times and is believed to have resulted from delayed spawning in this area. Delayed spawning has been reported for capelin from the Newfoundland region (Templeman 1948) and the Barents Sea (Prokhorov 1965). Olsen (1968), Prokhorov (1968), and Templeman (1948) attribute this late spawning to small females. Bailey et al. (1977b) collected small mature females in the study area well after the main period of spawning in 1974 and 1975. The mean length of yolk-sac larvae collected in August 1975 (5.3 mm) was significantly less ($P < 0.01$) than that of larvae collected during the normal spawning period (5.9 mm). This observation is also consistent with the findings of Baranenkova and Khokhlina (1970) for late spawning capelin and follows the theoretical predictions of Ware (1975) concerning egg and larval sizes in relation to spawning times in fish.

Distribution, Abundance, and Transport of Larvae

Capelin totally dominate the larval fish fauna of the St. Lawrence estuary from Île aux Coudres to Pointe des Monts during June to October and were the dominant larval form in the northwestern Gulf from Pointe des Monts to Sept-Îles when this area was sampled in late June 1975. West of Île aux Coudres, the approximate limit of the saltwater wedge (Greisman and Ingram personal communication), *O. mordax* replaced capelin as the dominant larval form. This change in species dominance was abrupt, suggesting differences in habitat preference or strong competition for available resources.

Seasonal patterns of abundance of capelin larvae in different regions of the estuary and northwestern Gulf of St. Lawrence are determined by a combination of the location and sequence of spawning and hatching in the area, and by subsequent drift caused by prevailing water movements. In the study area spawning occurs along the north shore from Île aux Coudres in the

TABLE 7. Comparisons of abundances (geometric mean of larvae/100 m^3 filtered) and standard lengths (mean, standard deviation) in millimetres of larvae in day–night samples from 24-h stations 1, 2, and 3 (Fig. 1), 1975.

Sampling depth (m)	No. of positive tows			Day	Night	Significance of differences
	Day	Night				
			Station 1, June 3–4			
0–4	4	2	Abundance	3474.0	1259.0	$P>0.05$
			Length	5.5(0.42)	5.5(0.35)	$P>0.05$
4–20	4	2	Abundance	660.7	467.8	$P>0.05$
			Length	5.4(0.42)	5.3(0.38)	$P>0.05$
18–52	4	1	Abundance	21.4	4.0	$P>0.05$
			Length	5.0(0.52)	5.1(0.36)	$P>0.05$
			Station 2, Aug. 4–5			
0–14	3	2	Abundance	44.3	34.5	$P>0.05$
			Length	5.4(0.50)	5.3(0.33)	$P>0.05$
9–38	2	1	Abundance	13.8	13.5	$P>0.05$
			Length	5.3(0.59)	5.6(0.41)	$P>0.05$
24–93	2	2	Abundance	1.2	7.7	$P<0.01$
			Length	5.5	5.1(0.55)	
			Station 3, Sept. 15–16			
0–46	4	2	Abundance	2.4	2.4	$P>0.05$
			Length	26.3(6.74)	29.9(3.51)	$P>0.05$
44–100	2	0	Abundance	1.4	0.0	
			Length	29.3(1.88)	—	
73–136	0	0	Abundance	0.0	0.0	
			Length	—	—	

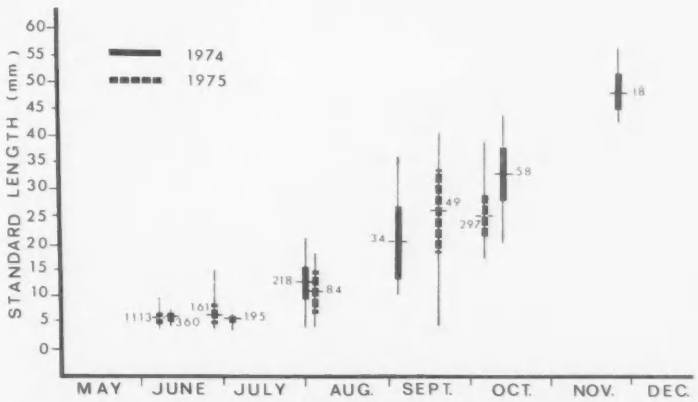

FIG. 7. Growth of capelin larvae in the St. Lawrence estuary and northwestern Gulf, 1974–75. Narrow vertical line represents the range, horizontal line the mean, and thick vertical line 1 SD on each side of the mean.

upper estuary to Baie-Comeau in region II of the lower estuary and in the northwestern Gulf east of Port-Cartier. On the south shore, spawning is restricted almost exclusively to the upper estuary and region I of the lower estuary (Fig. 10A).

In June of 1974 and 1975 capelin larvae occurred in greatest abundance in the upper estuary and on the south shore of the lower estuary in region I reflecting the concentration of spawning in the area at this time. In both years larval abundances were very low, or larvae were absent, on the north shore of region I of the lower estuary

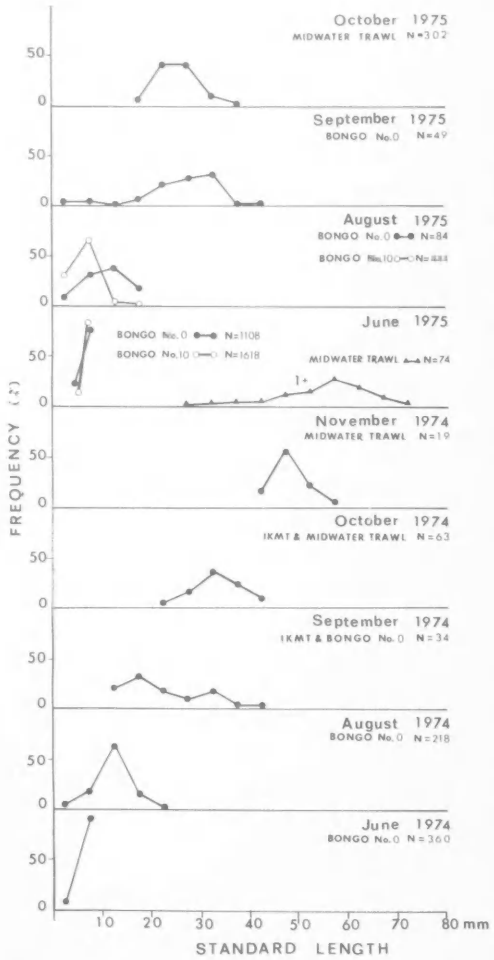

FIG. 8. Monthly length frequencies of capelin larvae in the St. Lawrence estuary and northwestern Gulf, 1974–75.

indicating that little or no spawning occurs in this region. The high concentration of larvae on the south shore in region I and the western portion of region II of the lower estuary apparently resulted from a combination of hatching of eggs spawned in these areas and from the drift of larvae into the area from upstream spawning sites. This occurs because larvae drifting out of the upper estuary are concentrated along the south shore by the general southward cross-channel flow in the area of the Saguenay River (Fig. 10B). Larval drift is clearly indicated by the occurrence of high densities of larvae along the south shore to the east of Rimouski, the approximate eastern limit of spawning areas on that shore (Fig. 10A). There is also evidence of a general northeasterly transport of larvae across the estuary in region II of the lower estuary by water currents in that area (Fig. 10B). The somewhat lower concentrations of larvae in the eastern portion of region II of the lower estuary in May 31–June 16 and June 25–29, 1975 must also have resulted from transport of larvae from upstream areas, since spawning has not been reported in this area.

The distribution of yolk-sac larvae in the upper estuary, the south shore of the lower estuary in region I, the north shore of region II, and the north shore of the western Gulf corresponds well with known spawning sites and dates for these areas. However, the occurrence of large numbers of yolk-sac larvae along a line extending from Rimouski (the approximate eastern limit of spawning on the south shore) to the vicinity of Pointe des Monts, the very high concentrations of yolk-sac larvae in the northeastern portion of this zone, and the occurrence of smaller numbers of these larvae east of this area during May 31–June 16, 1975 can only be explained by transport of larvae from spawning areas west of Rimouski on the south shore and east of Baie-Comeau on the north shore. The occurrence of yolk-sac larvae in region II of the lower estuary during June 25–29, 1975 must also have resulted from transport of larvae from upstream areas.

Further evidence for significant downstream transport of larvae and the role of currents in determining the distribution and character of larvae in particular areas is provided by the observation that the larger larvae collected during all sampling periods commonly occurred in the easternmost portion of the lower estuary and the northwestern Gulf, in spite of the fact that spawning and hatching occurred earliest in the upper estuary. This can be explained by the transport of the oldest larvae, which were spawned in the upper estuary as early as May 1 into the lower estuary and northwestern Gulf. R. G. Ingram (McGill University, personal communication) estimates drift time from Ile aux Coudres to Pointe des Monts to be approximately 4 wk. This corresponds well with our hypothesis.

The occurrence of small numbers of large larvae in the northwestern Gulf between Pointe des Monts and Sept-Iles during June 26–29, 1975 at the peak of the hatching in that area and coincident with a high percentage of yolk-sac larvae indicates that larvae may be transported considerable distances by water currents. A similar phenomenon occurred north of Anticosti Island during July 31–August 4, 1975. The maximum size of the large larvae collected near

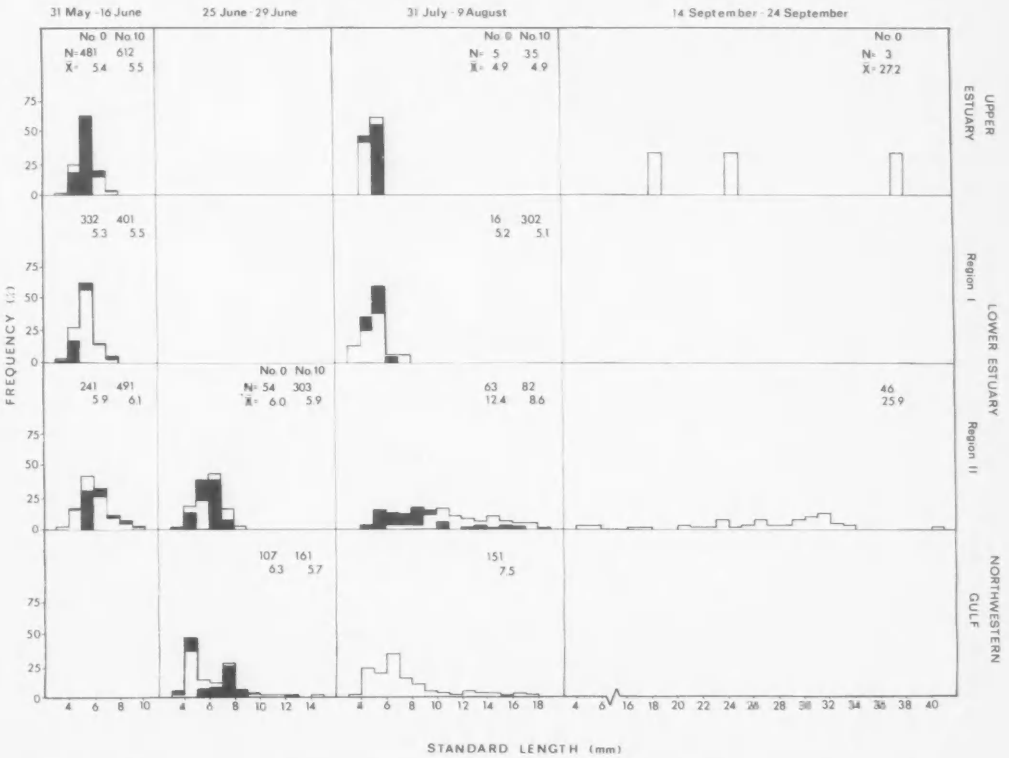

FIG. 9. Length-frequency distribution of capelin larvae collected in the St. Lawrence estuary and northwestern Gulf, 1975. Closed histograms = No. 10 net, open histograms = No. 0 net.

Anticosti Island at this time was similar to that of larvae collected in the lower estuary, but was much longer than that of the yolk-sac larvae in the same collections that were apparently the result of hatching in the sampling area.

This general pattern of downstream drift results in the virtual exclusion of larvae from the upper and lower estuary by September (99.99% reduction June–September 1975) and their concentration in the northwestern Gulf, west of Anticosti Island during October 1974 and 1975 and November 1974.

The decline in abundance of larvae in the estuary between June and September was, no doubt, also influenced by mortality. It is unlikely, however, that mortality alone accounted for this change because no similar reduction occurred in the northwestern Gulf.

Larvae transported to the northwestern Gulf may be retained there by a large counterclockwise gyre which occurs between Anticosti Island and Pointe des Monts (El-Sabh 1976). This area is within the region of maximum primary production in the Gulf of St. Lawrence (Steven 1971) and may serve as an important feeding area for capelin. Bailey et al. (1977b) reported juvenile and adult capelin were concentrated in this same area in October 1974 and 1975 and in November 1974.

The period of maximum larval abundance in the St. Lawrence estuary (late May–June) coincides with the annual maximum of primary production in this area. Furthermore, the smaller second peak of hatching occurring late July–early August corresponds to a smaller midsummer peak in primary production (Steven 1974; Sinclair et al. 1976). This link between spawning and production cycles has been reported for a number of other temperate species (Cushing 1975) and may be important in maximizing survival.

LARVAL GROWTH RATES

Average daily growth rates of capelin larvae in the St. Lawrence estuary (mean 0.25, range 0.20–0.35 mm/day) were slightly higher than

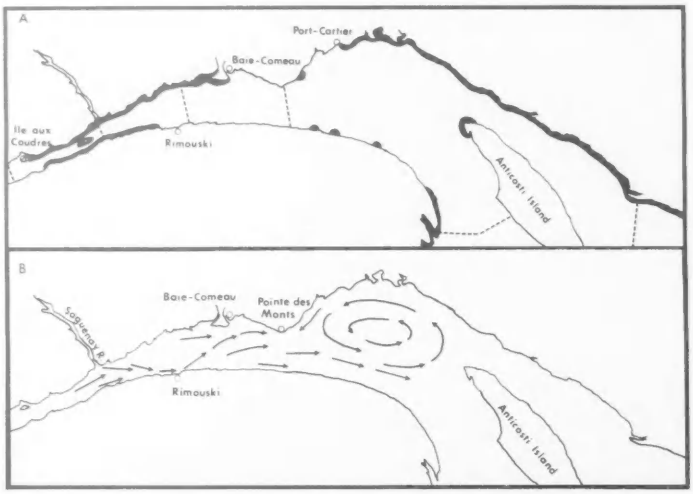

FIG. 10. Generalized representation of areas of capelin beach spawning activity, St. Lawrence estuary and northwestern Gulf (A) (after Parent and Brunel 1977), and generalized representation of major surface current patterns, St. Lawrence estuary and western Gulf (B) (after Forrester 1967, 1970; El-Sabh 1975, 1976; Greisman and Ingram personal communication).

those calculated from data provided by Magnusson (1966) for Icelandic capelin (mean 0.20, range 0.15–0.25 mm/day). These differences are not considered to be significant in view of possible biases resulting from differences in gear type, duration of the growth period observed, and sampling times between the two studies.

Bailey et al. (1977a) provided strong evidence indicating that the larval check ring described by Templeman (1948, 1968) is, in fact, the first annulus. This agrees with Templeman's reevaluation (1968) of capelin ages based on back-calculations from otoliths. Daily rates calculated from data provided by Templeman (1968) for 1st yr growth in the Newfoundland and Labrador areas (range 0.18–0.25 mm/day), when corrected for this discrepancy, also correspond with the rates observed in our study. The length of capelin larvae collected in the northwestern Gulf during November 23–25, 1974 at the approximate end of their first growing season ranged from 43 to 56 mm SL (mean: 48.4 mm SL, 53 mm TL). This is larger than corrected estimates for capelin from the Greenland, Newfoundland, and Labrador areas (Templeman 1968); however, some positive bias in the mean length of larvae from the northwestern Gulf is believed to have occurred because of selection for larger larvae by the Boris trawl.

Acknowledgments

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Seasonal and Vertical Distribution and Growth of Juvenile and Adult Capelin (*Mallotus villosus*) in the St. Lawrence Estuary and Western Gulf of St. Lawrence[1]

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The distribution of juvenile and adult capelin (*Mallotus villosus*) in the estuary and western Gulf of St. Lawrence was similar in 1974 and 1975. Juvenile (age 1 and 2) capelin were largely restricted to the western Gulf, which appears to serve as a nursery area for larval and juvenile capelin, throughout the summer and fall. During the summer and early fall capelin occurred in small schools which were widely distributed over most of the western Gulf. Concentration of juvenile and adult capelin into dense schools and their movement into the lower estuary in November were interpreted as the initiation of a contranatant migration to the spawning areas in the estuary. Spawning occurred first in the upper estuary (April–May) and was progressively later in the lower estuary and western Gulf. Postspawning mortality was high and resulted in a rapid decline in the abundance of adult capelin in the estuary and western Gulf following spawning. A concentration of juvenile capelin in and near the mouth of the Saguenay River throughout the year was apparently supported during the late summer by zooplankton production in the river. These fish, in turn, contribute heavily to the food resource of a large population of beluga whales that summer in the area. Capelin from the study area reach a greater mean size at the end of the 1st yr than do those from Newfoundland, Labrador, and Greenland. Subsequent growth was similar to that of stocks from southern Newfoundland but was faster than in stocks from northern Newfoundland and Labrador. Capelin populations in the estuary and western Gulf matured earlier and had younger mean ages than those from other areas in the Canadian Atlantic.

Key words: capelin, *Mallotus villosus*, Gulf of St. Lawrence, seasonal distribution, vertical distribution, growth, spawning

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La distribution des jeunes capelans (*Mallotus villosus*) et des adultes dans l'estuaire et le golfe Saint-Laurent occidental était identique en 1974 et 1975. Les jeunes (âges 1 et 2) étaient confinés en grande partie dans le Golf occidental, qui semble être une région d'alevinage pour les larves aussi bien que pour le jeunes capelans, durant tout l'été et l'automne. En été et au début de l'automne, le capelan se rencontre en très petits bancs répartis dans presque tout le Golfe occidental. Nous interprétons la concentration des jeunes et des adultes en bancs serrés et leur migration dans le bas Estuaire en novembre comme étant le début d'une migration à contre-courant vers les lieux de ponte dans l'Estuaire. La fraie a lieu d'abord dans le haut Estuaire (avril-mai) et progressivement plus tard dans le bas Estuaire et le Golfe occidental. La mortalité d'après la fraie est élevée et entraîne un déclin rapide de l'abondance des capelans adultes dans l'Estuaire et dans le Golfe occidental. Une concentration de jeunes capelans à l'embouchure du Saguenay à l'année longue semble être alimentée en fin d'été par la production de zooplancton dans le fjord. Ces poissons, à leur tour, constituent une abondante ressource alimentaire pour une grande population de dauphins blancs qui passe l'été dans la région. Le capelan de la région sous étude atteint une longueur moyenne plus grande à la fin de la première année que le capelan de Terre-Neuve, du Labrador et du Groenland. La croissance subséquente est semblable à celle des stocks du sud de Terre-Neuve, mais est plus rapide que celle des stocks du nord de Terre-Neuve

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et du Labrador. Les populations de capelans de l'Estuaire et du Golfe occidental atteignent la maturité plus tôt et sont en moyenne plus jeunes que celles qui fréquentent les autres régions de l'Atlantique canadien.

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CAPELIN (*Mallotus villosus*) constitute a significant portion of the diet of a large number of marine fish, mammals, and birds in Canadian Atlantic waters (Fig. 1). The recent dramatic increase in exploitation of this species (Winters 1975; Anon. 1976) has generated considerable concern for the continued viability of capelin stocks and for the possible impact of a reduction in their numbers on their predators (Winters 1975; Carscadden 1976; McCracken and Macdonald 1976). In spite of the importance of this species, knowledge of the ecology of capelin in Canadian waters is limited mainly to stocks occurring in waters around Newfoundland and Labrador and on the Grand Banks (see Carscadden 1976). Prior to 1975, virtually nothing was known of its ecology in the Gulf of St. Lawrence except for Templeman's (1948) study of biological characteristics of capelin from the Gulf waters immediately adjacent to Newfoundland. Recently, Hinds (1975) investigated the distribution and abundance of capelin in relation to its potential for exploitation there, and Parent and Brunel (1977) described the timing of spawning activity at various locations. Jacquaz et al. (1977) have reported on the ecology of larval capelin in the estuary and western Gulf, and Sharp et al. (1977) have commented on the possible existence of distinct spawning stocks there. This paper reports on the ecology of juvenile and adult capelin in this area.

Materials and Methods

Eight sampling cruises were conducted between May and December 1974 and between April and October 1975 in an area extending from Île aux Coudres in the St. Lawrence estuary to Natashquan on the north shore of the Gulf of St. Lawrence and to the entrance of Baie des Chaleurs in the southern Gulf (Fig. 2).

The principal aim of the first cruise (74008, May 28–June 21, 1974) was to determine the distribution and abundance of spawning capelin and as a result, searching was conducted mainly in shallow water areas nearshore. In all subsequent cruises transects were arranged to allow the most complete coverage of both deep and shallow waters in the time available (Fig. 3, 4). Survey effort was greatest between sunrise and sunset (approximately 75% of searching time).

During all cruises vertical echo sounders were employed to search for capelin (searching depth ranges of 0–119 and 0–238 m). Side scanning sonar was available for cruises 74008, 74019, 74051, and 75007 on the vessel *E. P. Le Quebecois*. Beginning with cruise 74051 (October 1–18, 1974) time and location marks were regularly recorded on the sounding paper. These, together with trawl catch data, were used to plot capelin distribution and estimate relative abundance. On three occasions (June 1974, August and October 1975) the day–night activity patterns of capelin were followed with an echo-sounder equipped vessel.

When fish were detected, trawling was conducted at the appropriate depth. The length of tows was generally restricted to <30 min to ensure the sampling of discrete groups of fish. The principal sampling gear used was a modified Boris midwater trawl (Bergeron 1973) equipped with a headline transducer (Boudreau 1973). In shallow water, samples were occasionally taken with a 4.5-m (16-ft) otter trawl set from a skiff. A small number of samples were taken by hand on spawning beaches. Small catches were weighed and the weight of large catches was estimated. A subsample of approximately 200 fish was randomly selected from each catch and examined fresh for length frequency and sex composition. Stage of maturity was determined for each fish in the sample. Total length (TL, in millimetres) was measured from the anterior tip of the mandible to the tip of the lower lobe of the caudal fin with this lobe manipulated to extend directly backward (Templeman 1948). Stage of maturity was deter-

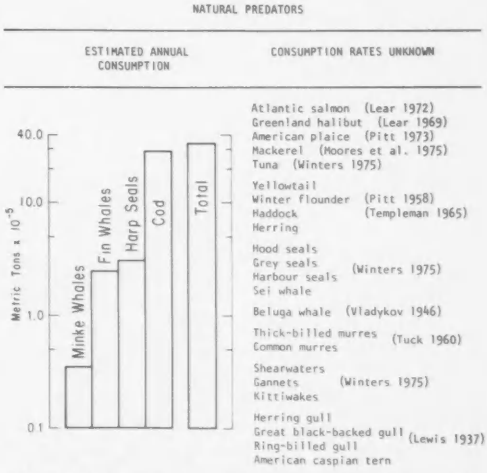

FIG. 1. Estimated annual consumption of capelin by four major predators (Winters 1975) and other known major predators for which no annual estimates of consumption are available.

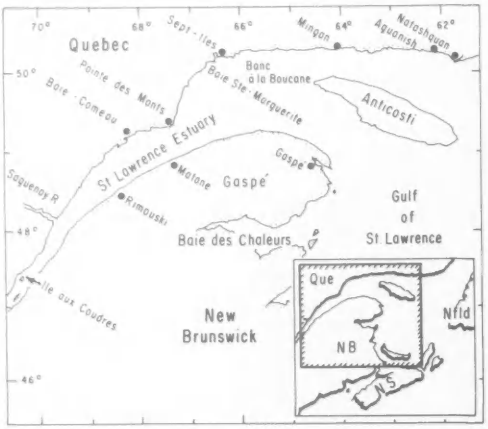

FIG. 2. St. Lawrence estuary and western Gulf of St. Lawrence with important localities mentioned in the text.

mined by gross examination of the gonads and secondary sexual characters. A second subsample of at least 50 fish was frozen and later aged using the method of Bailey et al. (1977). Frozen lengths were corrected to fresh lengths for growth analyses (Bailey et al. 1977). Spawning areas of Natashquan were sampled for eggs with a bottom grab during cruises 75007 (June 5–15, 1975) and 75019 (July 24–August 5, 1975).

At most sampling stations, and occasionally between stations, surface temperature and salinity were recorded. A bathythermograph (BT) or a salinity–temperature–depth recorder (STD) cast was also made to record salinity and/or temperature of the water column.

In this paper the term juvenile refers to fish in age-groups 1 and 2, maturing fish to capelin predominately age 2+ that would spawn the following spring (based on examination of gonads), and recovering adults to fish that had spawned the spring prior to capture.

Results

SEASONAL DISTRIBUTION

The distribution of capelin in the estuary and western Gulf in 1974 and 1975 was similar. At the time of the earliest cruise in this area (cruise 75001, April 29–May 14, 1975) capelin were abundant along the north shore from Île aux Coudres to and around the western tip of Anticosti Island but were less abundant on the south shore (Fig. 4A). In the estuary (Île aux Coudres to Pointe des Monts) catches were composed of approximately equal numbers of ripe and spent females and smaller numbers of ripe males and 2-yr-old juveniles. One exception to this general pattern was observed near the mouth of the Saguenay where 66% of the catch was 1- and 2-yr-old capelin. Local residents indicated that spawning had been occurring on beaches in the Ile aux Coudres area since mid-April. East of Pointe des Monts, in the western Gulf, catches contained 1- and 2-yr-olds and maturing fish only and these were frequently taken in the same trawl. The only exception to this pattern was at Baie Sainte-Marguerite where predominantly ripe fish were captured (Fig. 4A). A survey of the beach at Sept-Îles near Baie Sainte-Marguerite on May 11–12 revealed the presence of large schools of males but no indication of spawning.

During June of 1974 and 1975 (cruises 74008, May 28–June 21, 1974 and 75007, June 5–15, 1975) capelin were again observed in the estuary, along the north shore of the western Gulf to Natashquan, and off the eastern tip of the Gaspé Peninsula. No survey was conducted along the south shore of the estuary and western Gulf from Rimouski to Gaspé because little or no capelin spawning occurs in this area (Parent and Brunel 1977) (Fig. 3A, B, 4B). West of Pointe des Monts, in the estuary, the abundance of capelin was greatly reduced from the April–May levels and catches contained predominantly spent females with small numbers of spent males. A few ripe females were captured on the south shore of the estuary near the Saguenay and near Pointe des Monts in early June of 1974 and 1975. East of Pointe des Monts to Aguanish the catches contained a mixture of ripe and spent males and females in various proportions. Some mixed schools of 1- and 2-yr-old juveniles and spent capelin were sampled west of Mingan and off the eastern tip of the Gaspé Peninsula. Schools of capelin occurring offshore between Anticosti Island and the north shore were composed almost exclusively of juveniles. At Natashquan male and female capelin were collected in mixed schools located further offshore on the bottom at depths of 10–40 m on June 17–19, 1974 and June 10–11, 1975. Bottom samples obtained coincident with the trawls in 1975 contained large numbers of capelin eggs, indicating that offshore spawning was occurring. Bottom water temperatures were 4–5.5°C at the offshore spawning sites, as compared to 8.5°C near the beach. No eggs were found on the beaches adjacent to these spawning areas.

The reduction in abundance of capelin in the estuary noted in June 1974 and 1975 was even more pronounced during cruises 74019, June 28–July 7, 1974, and 75019, July 24–August 5, 1975 (Fig. 3C, 4C). During the 1974 cruise, which was restricted to the estuary, large concentrations of 1- and 2-yr-old juvenile capelin were observed and sampled near the mouth of the Saguenay River and near Pointe des Monts (Fig. 3C).

Apart from these, only three small schools of spent adults were detected. During the 1975 cruise very few capelin were observed anywhere along the cruise track. In the few cases where aggregations sufficiently large to allow sampling were detected, the collections contained small numbers of large juveniles, sometimes mixed with recovering spent adults.

In October (cruise 74051, October 1–18, 1974, and 75031, September 29–October 13, 1975) capelin were distributed throughout most of the western Gulf, but no large aggregations were observed (Fig. 3D, 4D). Catches from throughout the area contained mainly maturing capelin (age 2+) with small numbers of recovering adult fish that had spawned the previous spring. All fish captured at this time were feeding heavily. In both years capelin were virtually absent from the estuary except for large concentrations of 1- and 2-yr-old fish located near the Saguenay River (Fig. 3D, 4D).

In November (cruise 74056, November 20–December 2, 1974) schools, composed mainly of juvenile capelin mixed with small numbers of recovering adults, continued to be dispersed throughout the western Gulf. In contrast to October, large schools of 1- and 2-yr-old juvenile and recovering adult capelin occurred along both shores of the lower estuary. No capelin schools were detected in the area of the Saguenay at this time. However, severe weather conditions restricted sampling effort there.

Vertical Distribution

The maximum depth of occurrence of capelin in the study area was 100 m, but the majority of the capelin schools recorded occurred at depths of <50 m which was the approximate upper limit of the cold-water (0°C) layer. Day–night monitoring of large concentrations of spent and mature female capelin at Aguanish on June 20–21, 1974, and of juvenile male and female capelin at Banc à la Boucane on August 1–2, 1975, and Rochers du Saguenay October 1–2, 1975, revealed consistent patterns of diurnal vertical migration and aggregation. During periods of darkness capelin were evenly dispersed near the surface at depths of 0–20 m. During daylight hours they were deeper in the water column and were most frequently concentrated into discrete schools (Fig. 5).

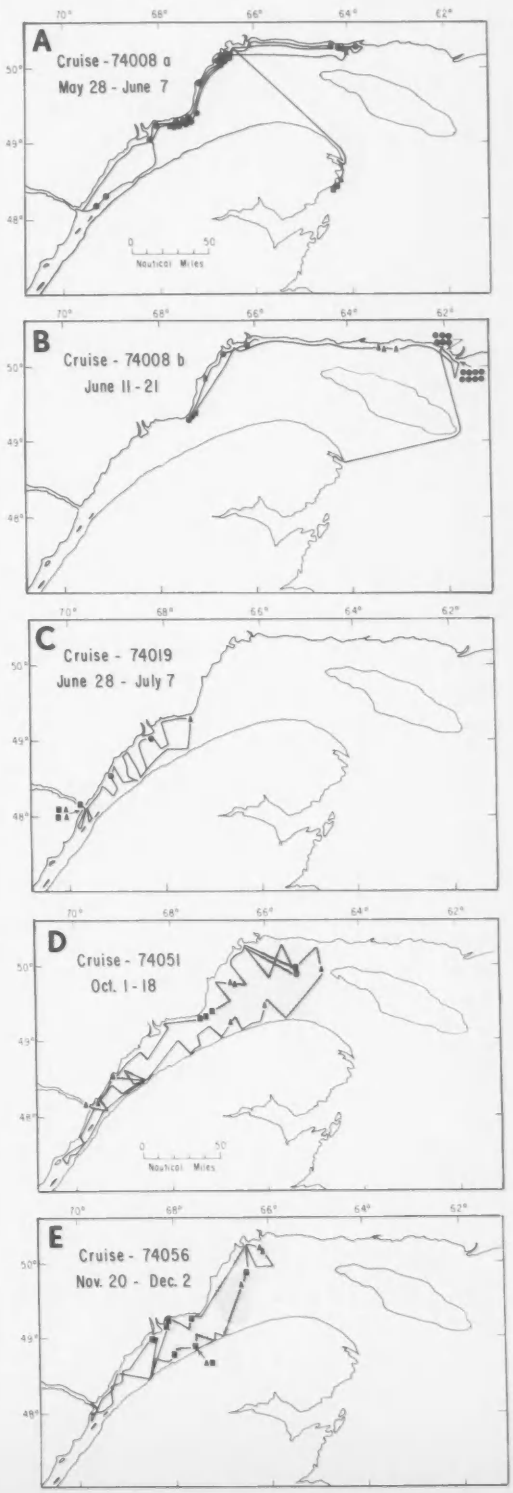

FIG. 3. Survey track, sampling stations, and catches (● adults, ▲ juveniles, ■ mixed) for each cruise during 1974. Shaded area indicates presence of capelin based on echo-sounder recordings. Sounder records not obtained on tracks A, B, C.

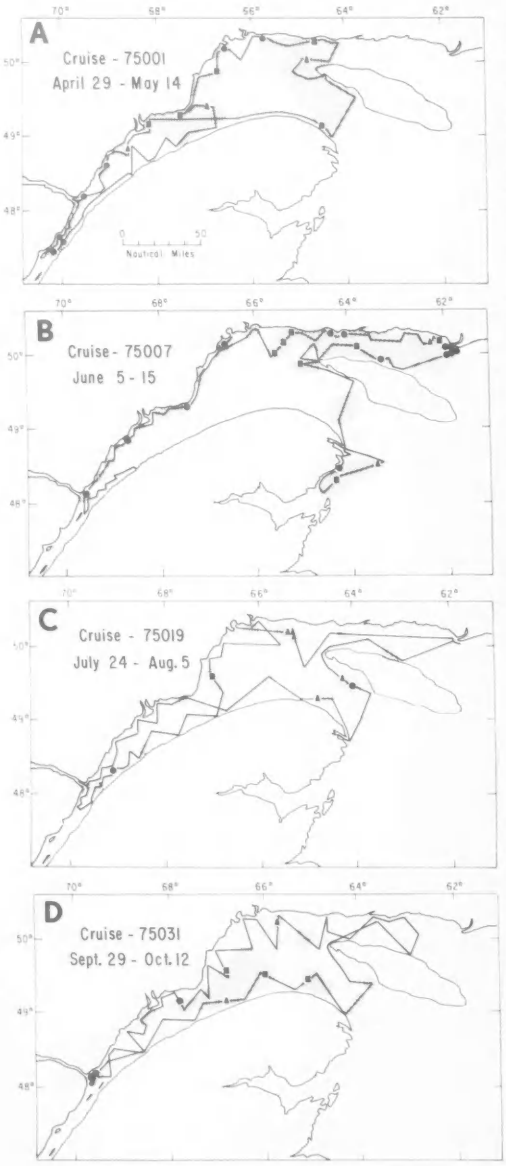

FIG. 4. Survey track, sampling stations, and catches (● adults, ▲ juveniles, ■ mixed) for each cruise during 1975. Shaded area indicates presence of capelin based on echo-sounder recordings.

With one exception, there did not appear to be a significant difference in the vertical distribution of adult and juvenile capelin (Table 1) or of different age-groups. This exception occurred in November 1974 when capelin detected in the lower estuary in the region of Baie-Comeau and Matane (Fig. 3E) were observed to be schooling in two distinct layers (Fig. 6). Discrete sampling of the two layers revealed that the top layer contained 80–90% 1-yr-olds, while the bottom layer 56–70% 2-yr-olds, the balance being predominantly 3- and 4-yr-olds mixed with a small number of 1-yr-olds.

POPULATION PARAMETERS

Average total lengths (by sex and age) of all capelin sampled throughout the study, back-calculated lengths at each otolith annulus, and von Bertalanffy growth equations based on these data are plotted in Fig. 7. The theoretical maximum length L_∞ for males and females was estimated from the equations to be 225 and 200 mm, respectively. This is in reasonable agreement with the maximum lengths observed in our catches.

Some evidence of annual differences in growth was observed. The ranges of mean lengths of 2- and 3-yr-old male and female capelin collected during the cruises in 1974 were consistently greater than those of fish collected at equivalent times during 1975:

	Males		Females	
	2+	3+	2+	3+
1974	137–162	182–186	139–152	167–175
1975	106–142	152–179	115–132	159–162

No significant difference was observed in the average lengths of 4+ fish in the 2 yr, possibly because these fish were approaching L_∞ and hence growth was less affected by interyear differences.

In the combined 1974 and 1975 catches 90.4% of the male capelin was 3 yr old; the remainder (9.6%), 4 yr. The age distribution of females was age 2, 2.4%; age 3, 80.2%; age 4, 16.6%; age 5, 0.8%. The mean ages of mature male and female capelin did not differ. Larger females spawned earliest in all regions as evidenced by the fact that the mean length of spent females was consistently larger than that of ripe females (Table 2). This pattern was also evident in the declining average length of ripe females sampled over a period of 2 wk near the spawning beach at Baie Sainte-Marguerite during May–June 1974.

Date	Number	Mean length (mm)	SD
May 28	335	165.3	11.02
May 30	218	167.1	10.5
June 4	117	163.4	13.1
June 12	69	160.0	15.2

Templeman (1948) and Winters (1974) reported

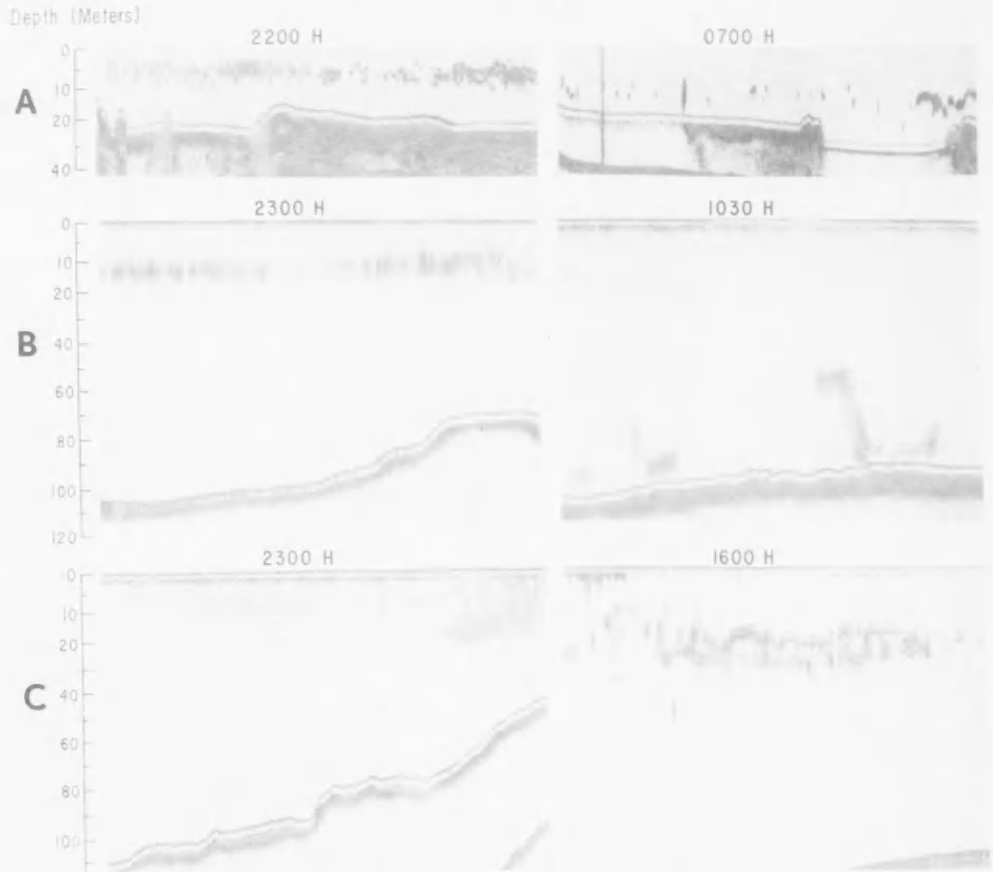

FIG. 5. Day–night variation in vertical distribution of capelin schools. A, Aguanish, June 20–21, 1974; B, Banc à la Boucane, August 1–2, 1975; C, Rochers du Saguenay, October 1–2, 1975.

similar findings for capelin spawning in the Newfoundland area.

A total of 3997 fish from 25 trawl collections made in October–November 1974 and April–October 1975, which contained only juvenile and maturing capelin, were examined for sex. Males were less abundant than females in 18 of the 25 collections. The ratio of males to females in the total sample was 0.45:0.55.

In 1974 and 1975 postspawning mortality was very high in males and females. This was evident in the rapid decline in the percentage of 3- and 4-yr-old fish in the catches after June (Table 3) and by the low numbers of 4- and 5-yr-old fish in the stocks (Table 2). Instantaneous mortality rates (Ricker 1975) for age-groups 3 and 4 were

	Age 3	Age 4
Males	2.24	—
Females	1.58	4.8

Relative catch per unit effort for capelin in the estuary and western Gulf in 1974 and 1975 exhibited similar seasonal patterns (Fig. 8), although the abundance of capelin was lower in the spring of 1975.

Discussion

SEASONAL DISTRIBUTION

Jacquaz et al. (1977) indicate that drift of young-of-the-year capelin spawned in the estuary and northwestern Gulf of St. Lawrence results in

TABLE 1. Fishing depth and temperature ranges for adult and juvenile capelin sampled in the estuary and western Gulf of St. Lawrence, 1974 and 1975.

Sampling period	No. of stn.	Adult		Juvenile		Temp range of water column (°C) at depths		
		Fishing depth range (m)	Temp range (°C)	Fishing depth range (m)	Temp range (°C)	15 m	30 m	50 m
1974								
May 28–June 7	22	7–28	5.0–9.5	7–25	6.0–8.0	—	—	—[b]
June 11–21	20	11–73	0.0–8.0	9–20	3.5–8.0	1.1–5.7	0.5–2.7	−0.3–0.3
June 28–July 7	10	18–46	0.5–6.0	11–40	0.0–5.0	1.2–6.1	0.4–3.2	−0.6–2.9
Oct. 1–18	15	—[a]	—[a]	8–27	1.0–3.8	2.2–5.5	−0.1–5.0	−0.2–3.8
Nov. 20–Dec. 2	11	—[a]	—[a]	27–28	−1.0–1.5	0.1–1.8	0.2–1.7	0.3–1.4
1975								
Apr. 29–May 14	17	9–22	1.8–4.3	11–28	0.0–2.5	−0.2–3.4	−0.3–1.2	−0.9–0.1
June 5–15	16	9–36	1.0–6.5	16–91	0.0–5.5	2.4–6.7	0.5–5.5	−0.2–1.9
July 24–Aug. 5	7	13–22	2.5–13.5	13–25	2.5–11.0	1.6–13.5	0.2–9.5	−0.3–7.0
Sept. 29–Oct. 13	9	—[a]	—[a]	5–45	1.0–9.2	1.1–7.3	0.5–7.0	0.0–4.2

[a]Small numbers mixed with juvenile schools.
[b]Not available.

FIG. 6. Echo-sounder record of capelin schools with temperature profile of the water column near Matane on November 23, 1974.

their concentration, during the fall, in the northwestern Gulf between Pointe des Monts and Anticosti Island. They conclude that this area, which is within the region of maximum primary production in the Gulf of St. Lawrence (Steven 1971), may serve as an important feeding area for young-of-the-year capelin. The virtual absence of 1-yr-old capelin from the estuary, and their distribution throughout the western Gulf over the period of our study, correspond with the expected distribution of this age-group based on their findings and suggest that the region is also important as a feeding area for 1-yr-old fish. Two-yr-old fish were more widely distributed within the study area but during the summer and fall they too were largely restricted to and presumably actively feeding in the western Gulf.

The movement of large concentrations of 1- and 2-yr-old juveniles, together with recovering adults, into the lower estuary in November may represent the beginning of a contranatant migration to the spawning areas in the upper estuary. Barents Sea capelin are reported to concentrate in November to begin the migration which ter-

TABLE 2. Mean total length of adult female capelin during spawning times at different regions in the estuary and western Gulf of St. Lawrence. *$P < 0.05$; **$P < 0.01$; NS = not significant.

Period	Region	No.	Ripe		Spent		t-test between maturities
			No.	Mean length (mm)	No.	Mean length (mm)	
1974							
May 28–June 21	Upper estuary	109	7	163.3	102	173.5	*
	Lower estuary	3695	142	161.6	3553	171.2	**
	Sept-Îles	2217	779	164.7	1438	170.7	**
	Mingan	293	145	168.8	148	170.1	NS
	Natashquan	3266	1185	165.9	2081	172.5	**
1975							
Apr. 29–May 14	Upper estuary	348	198	162.7	150	171.1	**
June 5–15	Mingan	998	563	150.8	435	160.7	**
	Natashquan	640	390	162.2	250	165.1	**

TABLE 3. Percent age composition of capelin from the estuary and western Gulf of St. Lawrence, 1974 and 1975.

Sampling period	Sex	Sample size	% in age-group				
			1	2	3	4	5
1974							
May 28–June 7	males	112	0	0.9	89.0	10.1	0
	females	232	0	1.6	82.0	16.1	0.3
June 11–21	males	80	0	0	97.7	2.3	0
	females	158	0	0.6	87.6	11.6	0.2
June 28–July 7	males	8	87.5	12.5	0	0	0
	females	91	50.5	1.6	40.7	7.1	0
Oct. 1–18	males	151	49.7	44.3	5.3	0.7	0
	females	221	56.3	33.3	8.1	2.3	0
Nov. 20–Dec. 2	males	82	62.0	36.1	1.9	0	0
	females	137	53.0	35.8	10.4	0.7	0
1975							
Apr. 29–May 14	males	86	0	66.3	31.4	2.3	0
	females	113	0	45.1	31.9	23.0	0
June 5–15	males	58	0	15.5	72.4	12.1	0
	females	127	0	19.7	74.0	4.7	1.6
July 24–Aug. 5	males	37	2.7	94.6	2.7	0	0
	females	59	0	96.6	3.4	0	0
Sept. 29–Oct. 13	males	48	16.7	81.2	2.1	0	0
	females	49	8.2	91.8	0	0	0

minates in spawning in February through April (Prokhorov 1965; Olsen 1968). Most of the Gulf and estuary is not navigable from December to April and direct observation of capelin movement is not possible at this time. However, Sergeant (1973) reported capelin comprise over 85% of the diet of harp seals (*Pagophilus groenlandicus*) in the estuary near the Saguenay during January–April. This suggests that the movement into the estuary, first noted in November, may continue throughout the winter. This would explain the presence of large numbers of 2-yr-old and adult capelin in the upper estuary in April. A similar movement toward spawning areas in the western Gulf presumably occurs; however, the proximity of these spawning areas to the feeding area and the later time of spawning there may result in a later initiation of this movement.

SAGUENAY RIVER POPULATION

A major exception to the pattern of seasonal distribution described above was year-round occurrence of juvenile capelin in and near the mouth

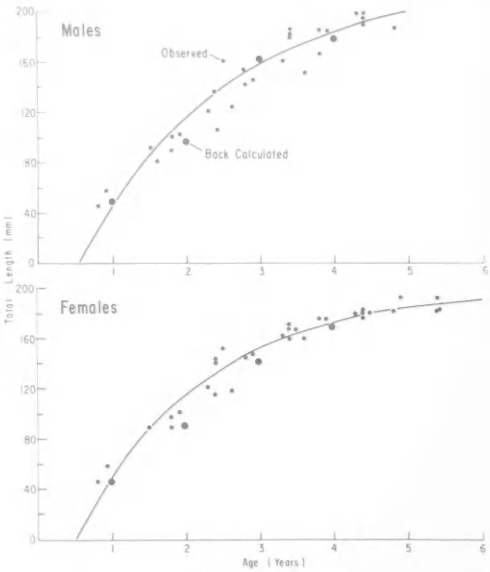

FIG. 7. Observed and back-calculated mean lengths of capelin sampled in the estuary and western Gulf during 1974 and 1975. Von Bertalanffy equations: males, $L_t = 225(1 - e^{-0.50(t-0.51)})$; females $L_t = 200(1 - e^{-0.58(t-0.32)})$.

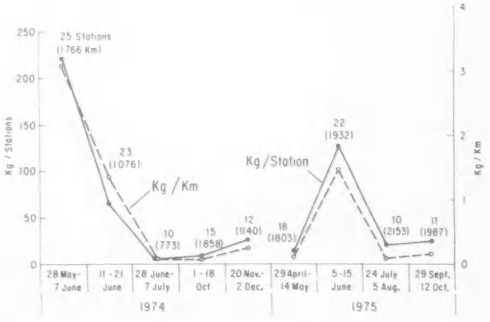

FIG. 8. Annual and seasonal variation in availability of capelin during 1974 and 1975 in the St. Lawrence estuary and western Gulf.

of the Saguenay River. Rainville and Lacroix (1976) report that zooplankton densities in the estuary near the Saguenay decline rapidly from 10–20 mg/m³ in June and early July to near zero in August and September. Jacquaz et al. (1977) report a similar pattern. However, in the lower Saguenay zooplankton biomass remains more stable, ranging between 2.5 and 7.5 mg/m³ from May to October (Rainville and Lacroix 1976). The relatively stable zooplankton biomass in the lower Saguenay may provide a food resource to support a limited number of juveniles in the area throughout the year. This population of capelin, in turn, contributes significantly to the food resource (Vladykov 1946) of the 500–1000 beluga whales (*Delphinapterus leucas*) that regularly occur in and near the mouth of the Saguenay during the summer (Sergeant and Brodie 1975).

Spawning and Mortality

At the time of our earliest sampling in the upper estuary near Île aux Coudres (April 29, 1975), spawning had already commenced because samples obtained at that time contained large numbers of spent females. The virtual absence of spent fish in the lower estuary and western Gulf at this time, and the large numbers of maturing adults in these areas, suggest spawning occurs later there. Spent males and females first appeared in samples from the western Gulf in June. This temporal variation in spawning time agrees with that reported by Parent and Brunel (1977).

The heavy mortality experienced by 3- and 4-yr-old capelin following spawning results in the limited numbers of adult fish throughout the study area during the summer and fall. Mortality was greater in males than in females, probably because of their prolonged residence on the spawning beaches. High mortality following spawning and elevated mortality among males have been reported previously for capelin (Templeman 1948; Campbell and Winters 1973; Winters and Campbell 1974). Adults that survive spawning, together with the 2-yr-old fish that accompany them to the upper estuary, appear to move out of the region into the western Gulf during late June and July, since very few were captured there during the summer. While capelin were considerably more abundant in the western Gulf during the summer and early fall, they occurred only in very small schools which were widely dispersed over much of the area sampled. This dispersal, noted in both 1974 and 1975, may be related to feeding behavior. Eggers (1976) has suggested that prey consumption may be increased if schooling fishes disperse while feeding.

Vertical Migration

The pattern of diel vertical migration observed during day–night monitoring of discrete capelin concentrations in the estuary and western Gulf were similar to those observed for capelin in the Gulf of St. Lawrence (Hinds 1975), on the Grand Banks (Kovalyov and Kudrin 1973; Dragesund and Monstad 1973) and Labrador (Devold et al. 1972).

GROWTH

Growth rates of capelin in the study area were similar to those of populations spawning in southern Newfoundland and on the Grand Banks but were significantly faster than those reported for northern Newfoundland and Labrador (Winters 1974).

The slower growth observed in northern populations may result from differences in the length of the growing seasons and/or food availability there.

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Production and Population Dynamics of the Crayfish *Orconectes virilis* in Three Michigan Lakes[1]

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MOMOT, W. T., AND H. GOWING. 1977. Production and population dynamics of the crayfish *Orconectes virilis* in three Michigan Lakes. J. Fish Res. Board Can. 34: 2041–2055.

Fluctuations in mortality rather than in growth rates produced most of the year-to-year differences in biomass accumulation of three different populations of the crayfish *Orconectes virilis*. Yearly biomass changes resulted from density-dependent control of mortality and fecundity during certain portions of the life cycle. Density-dependent changes in mortality rates controlled population size for adults in all lakes and for young-of-the-year in two of the three study lakes. Growth rates were much less responsive to fluctuating densities. Disparity between the number of ovarian and attached eggs increased as density of age I+ crayfish increased. This provided a strong density regulator on fecundity. Differences occurred in the number of recruits produced by a brood stock that survive to the end of the first growing season in the various lakes. Yet the number of females surviving to reproductive age 2 yr later was strongly regulated. Strong population regulation produced two female recruits of breeding age for every two–six parental breeding females. The high biomass and production levels of crayfish discovered in West Lost Lake in 1962–63 also occurred in the other area lakes. Higher but variable levels of recruitment resulted in larger standing crops and production in West Lost lake. This resulted from less effective density controls on the mortality rates of younger age-groups in that lake. Despite great variation in biomass of from 46 to 213 kg/ha and annual production from 60 to 142 kg/ha, the annual turnover ratio of the biomass was found to vary only between 0.94 and 1.53.

Key words: crayfish, *Orconectes virilis*, population dynamics, annual production, Michigan lakes

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Les différences d'une année à l'autre dans l'accumulation de biomasse de trois populations différentes d'écrevisses *Orconectes virilis* sont dues à des fluctuations de mortalité plutôt que des rythmes de croissance. Les changements annuels dans la biomasse résultent du contrôle de la mortalité et de la fécondité, dépendant de la densité, durant certaines portions du cycle biologique. Des changements dépendant de la densité dans les taux de mortalité contrôlent la taille des populations adultes dans tous les lacs et celles des jeunes de l'année dans deux des trois lacs étudiés. Les rythmes de croissance sont beaucoup moins sensibles aux fluctuations de densité. La différence entre le nombre d'œufs ovariens et d'œufs fixés augmente à mesure qu'augmente la densité des écrevisses d'âge I+. Ceci constitue un bon agent de contrôle de la densité par la fécondité. On constate des différences dans le nombre de recrues produites par un stock reproducteur qui survit jusqu'à la fin de la première saison de croissance dans les divers lacs. Cependant, le nombre de femelles atteignant l'âge de la reproduction 2 ans plus tard est fortement contrôlé. Un contrôle rigide des populations produit deux recrues femelles sexuellement mûres pour chacune deux-six femelles reproductrices maternelles. Les niveaux élevés de biomasse et de production d'écrevisses découverts dans le lac West Lost en 1962–63 se sont produits également dans d'autres lacs. Des niveaux plus élevés mais variables de recrutement résultèrent en des biomasses et une production plus abondantes dans le lac West Lost. Ceci provint de contrôles de densité moins efficaces sur les taux de mortalité des groupes d'âge plus

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jeunes dans ce lac. En dépit de la grande variation dans la biomasse, soit de 46 à 213 kg/ha, et dans la production annuelle, soit de 60 à 142 kg/ha, on constate que le rapport de renouvellement annuel de la la biomasse ne varie que de 0.94 à 1.53.

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THE widely distributed pothole lakes of the northern lower peninsula of Michigan are highly transparent, yet contain little or no aquatic vegetation because of low concentrations of organic matter. Some lakes have a high percentage of marl in their sediments (Hale et al. 1903). These marl encrustations produced by various species of benthic algae cover rocks and other fixed objects within the epilimnion. Because phytoplankton production is typically low (Raymond 1937) benthic algae account for most of the fixed carbon photosynthesis (Hooper and Ball 1964). Production of other benthos, such as insects, is limited by lack of aquatic plants (Wohlschlag 1950; Berg 1949). Indeed, production in lakes similar to these is low for all trophic levels (Wetzel 1970). Yet in West Lost, the lake we initially studied, crayfish dominated the macrobenthos, with their annual production fourfold that of other macrobenthos (Momot 1967a). This is probably because of the polytrophic diet of crayfish and their ability to utilize allochthonous sources of energy (Momot et al. 1977). The study was expanded to see whether the large populations found in West Lost were typical for other lakes in the general area. We also wished to determine whether high production rates found in West Lost were also to be found in the other lakes. Finally, we wanted to see whether high production rates wherever found were sustained over a period of several years.

Population dynamics of the crayfish *Orconectes virilis* (Hagen) in three pothole lakes were monitored over a 4-yr period, 1966–69. We report here on abundance, survival, reproduction, recruitment, annual production, and turnover ratios of the crayfish populations in these lakes. Most of the changes in the biomass and productivity of these populations will be shown to be the result of internal density-dependent processes operating on the fecundity and mortality rates rather than growth.

Description of Area

West Lost, North Twin, and South Twin are limestone sink lakes at the Pigeon River Fisheries Research Station in Otsego County, Michigan. They are morphometrically similar, small in area, subcircular in outline, and steeply cone shaped in form, lacking an inlet or outlet. Lake surfaces are 12–18 m below the surrounding terrain, and the basins have very steep slopes. These lakes, which are in close proximity of one another, range in area from 1.4 to 1.9 ha with mean depths of 7.3–8.5 m (Table 1). The shoal areas, defined as water <2.2 m in depth (the depth to which rooted aquatic vegetation extends), are narrow and make up only 16–20% of the total bottom. Predominant substrates are sand in the shoal area and outward to a depth of 3 m, organic matter out to 6 m, and pulpy peat in the profundal area. Logs are abundant in the littoral area and are marl encrusted in West Lost Lake. The limited submerged aquatic vegetation consists mainly of yellow lily, *Nuphar variegatum* Engelm. Most of the aquatic vegetation consists of the sedge *Carex aquatilis* var. *substricta*.

The lakes stratified thermally during the summers and winters of the study period from November 1965 to October 1969. Summer oxygen levels >3.9 mg/ℓ usually extended down into the thermocline to depths varying from 7 m (South Twin) to 9 m (North Twin). North Twin had the softest water and poorest conductivity of the three lakes, while West Lost had the hardest water and highest conductivity (Table 1). South Twin Lake was intermediate in these qualities. Tanner (1960) gives additional limnological information on these lakes.

Materials and Methods

The size of the adult crayfish population was estimated during the spring and summers of 1966–69. During these years population estimates of young-of-the-year crayfish were made in late summer. Because seining was impossible in the steep littoral zone, crayfish <20 mm in cephalothorax length were collected with long-handled dip nets 0.5 m in diam and measured, marked, and released along the shoreline. The entire circumference of the lake was sampled each time. Round, wire minnow traps were used to collect adults in each lake. The exact placement of traps is described in Momot and Gowing (1972). Traps were baited with remains of frozen fish and set for 24 h, then lifted and rebaited. After being processed (marked, measured, weighed, etc.), crayfish were released in the center of the lake. Crayfish were marked by removing the pleura. Clipping the pleura did not alter ecdysis but regeneration resulted in a slightly deformed pleuron. Marked animals retained this scar over winter.

Care was taken so that no recruitment occurred during each sample period. By sampling between adult molt periods growth and mortality are greatly reduced. Recruitment through growth did not occur. Recruitment by the addition of new members also did not occur, since young-of-the-year grew too slowly to enter the traps and were easily distinguished from adults because of their smaller size. The method

TABLE 1. Physical and chemical characteristics of three Pigeon River area lakes, 1966.

Lake	Surface area (ha)	Depth (m)		% shoal[a]	Avg total alkalinity	pH (mean)	Specific resistance (ohms-cm)[b]
		Max	Avg				
West Lost	1.4	13.1	8.5	20.1	134.0	8.0	4,156
North Twin	1.9	12.5	8.2	19.6	29.0	7.4	20,900
South Twin	1.6	10.1	7.3	16.5	65.0	7.8	8,400

[a]Water < 2.2 m.
[b]Corrected to 25°C.

used assured that (1) animals did not lose their marks during sampling and (2) all marked animals were easily recognized.

We assumed that marked and unmarked animals of the same sex have the same probability of capture. Though *O. virilis* has not been investigated, Morrissy (1973) showed that for *Cherax tenuimanus*, marked and unmarked crayfish were equally vulnerable to traps. There is no evidence which would cause us to suspect catchability being affected by the marking method in *O. virilis*. In an earlier paper (Momot 1967a) we successfully estimated a population of known size using the mark-and-recapture technique.

The successful use of the trapping method in estimating the population size of *O. virilis* depends on its widespread random movement in lakes. Various studies on orconectid crayfish have shown them to wander extensively (Camougis and Hichar 1959; Goellner 1943; Fielder 1972). Random dispersment allowing a mark-and-recapture estimate of population size has also been shown for *Astacus astacus* (Abrahamsson 1966; Abrahamsson and Goldman 1970) and for *Pacifasticus leniusculus trowbridgii* (Mason 1963). Hence, the wandering random movements of crayfish in lakes with uniform substrates make them amenable to mark-and-recapture estimates.

Population densities were estimated by the Schumacher-Eschmeyer formula (Ricker 1975). Confidence limits on estimates of adult densities usually fell within ±10% of the estimate; confidence limits on young-of-the-year usually fell within ±20% of the estimate (Table 2).

Age composition was determined from size-frequency graphs. Recaptures of crayfish marked the year before provided size-frequency graphs of known age animals for comparison. The use of arithmetic probability paper described by Hopkins (1966) also helped us to analyze length-frequency curves for age assessment. The slow growth of young-of-the-year and yearling *O. virilis* in these lakes enable us to apportion the population into size-groups with distinct modes (Momot 1967a). Extensive overlap between age-groups occurred only for age II and III crayfish. A plot of known age crayfish indicates the overlap to be about 20%. For these age-groups, at least 80% of the age II and III animals can be aged by inspection of the size-frequency polygons (Momot 1967a). Carapace length in millimetres was measured with vernier calipers along the dorsal midline from the tip of the rostrum to its posterior end and is the length measurement used throughout this paper.

Growth data were converted from length to weight units with the length–weight relationship given in Momot (1967a). Crayfish with complete sets of appendages were measured to the nearest millimetre and weighed to the nearest 0.1 g. Males and females do not differ in weight for a given size (Momot 1967a). The instantaneous rate of mortality (Z), after Ricker (1975), was derived from the population estimates. Mortality, calculated for all groups during the summers of 1966–69, was determined from population estimates stratified by age-groups. Age 0 crayfish were estimated by multiplying the sum of the attached-egg counts by the number of breeding females estimated to be present in the spring. Age 0.5 crayfish actually represent the number of young-of-the-year crayfish present in early fall (late August) that were hatched in June. Annual production was estimated using the Ricker (1975) method because growth and mortality trends were more nearly approximated by exponential functions.

During the spring (between May 15 and 31) of each year egg-bearing females were collected in shallow waters with a small hand-net. In early fall (between September 27 and October 8) of each year female crayfish were collected in minnow traps. All adult females were collected including those with no attached eggs. Ovaries were removed from fall-caught crayfish and all visibly developed eggs were counted. Eggs attached to the pleopods of crayfish caught in the spring were used to estimate attached-egg counts.

Characteristics of the Crayfish Populations

POPULATION SIZE

Each population estimate consisted of a series of individual estimates stratified by age-groups and lakes (Table 3). The numerical size and weight of each stock varied considerably from year to year because of differences in growth, reproduction, and natural mortality. These changes are most meaningful when considered with mean growth and mortality rates later on in the text.

GROWTH

Growth varied annually within any one lake (Table 4). Seasonal growth of crayfish occurred in stages related to periodic molting. Frequency

TABLE 2. Population estimates of crayfish during the summers of 1966, 1967, 1968, and 1969, excluding newly hatched young.

Year and lake	Sampling: No. of samples	Sampling: Sex, size-group (mm)	Sampling: No. marked	Sampling: Recapture rate (%)	Population estimate: No.	Population estimate: 95% CL
1966						
W. Lost	8	♂ + ♀ 10–20	14,158	20	54,996	52,702– 57,498
	13	♂ 24–33	6,360	38	22,304	17,667– 25,000
	13	♂ 34–45	434	60	703	587– 878
	20	♀ 24–36	2,753	32	9,056	8,203– 10,111
N. Twin	11	♂ + ♀ 10–20	1,323	3	44,959	26,378–160,666
	12	♂ 24–33	3,872	33	11,856	10,881– 13,140
	12	♂ 34–45	344	28	931	751– 1,236
	27	♀ 24–37	1,927	19	10,040	9,216– 11,049
S. Twin	13	♂ + ♀ 10–20	1,379	2	39,586	30,030– 59,880
	14	♂ 24–33	3,325	30	10,101	8,920– 11,918
	14	♂ 34–35	963	48	1,819	1,670– 2,002
	28	♀ 24–36	1,129	22	4,740	4,657– 4,828
1967						
W. Lost	20	♂ + ♀ 10–20	2,904	6	46,996	40,485– 54,054
	9	♂ 24+	3,769	61	4,902	4,409– 5,518
	31	♀ 21+	949	27	3,703	3,241– 4,319
N. Twin	19	♂ + ♀ 24+	995	3	37,243	25,773– 39,370
	9	♂ 24+	2,558	53	4,081	3,572– 4,759
	32	♀ 21+	1,120	12	4,366	3,865– 5,017
S. Twin	27	♂ + ♀ 10–20	1,240	4	36,245	20,883–142,857
	9	♂ 24+	2,707	61	4,085	3,439– 4,918
	32	♀ 24+	550	13	3,225	2,602– 4,240
1968						
W. Lost	13	♂ + ♀ 10–20	3,832	24	21,077	19,305– 23,255
	9	♂ 21+	2,620	38	4,733	3,987– 5,714
	22	♀ 20+	725	13	3,886	3,717– 5,104
N. Twin	26	♂ + ♀ 10–20	3,777	15	22,710	20,242– 25,906
	10	♂ 20+	4,413	48	7,354	5,973– 9,578
	18	♀ 18+	1,549	35	4,471	3,260– 6,035
S. Twin	10	♂ 18+	10,593	60	17,163	16,207– 18,281
	18	♀ 19+	2,301	26	8,356	7,336– 9,718
1969						
W. Lost	13	♂ + ♀ 10–20	3,467	6	64,154	57,471– 65,359
	8	♂ 24–33	3,199	54	5,492	4,932– 6,211
	24	♀ 20–37	779	14	4,321	3,741– 5,141
N. Twin	15	♂ + ♀ 10–20	3,040	10	47,194	34,246– 78,125
	10	♂ 21–45	2,646	50	5,108	4,098– 6,849
	17	♀ 19–39	1,018	18	3,959	3,334– 4,899
S. Twin	17	♂ + ♀ 10–20	1,439	6	32,096	28,011– 38,023
	7	♂ 34–42	3,299	67	5,037	4,564– 5,678
	28	♀ 22–36	485	19	3,098	2,543– 3,472

of molting of *O. virilis* in these lakes was such that after yearling males matured, the molt sequence was always the same; males molted twice and females once (Momot 1967a). Immatures molted up to 4 times a year (Aiken 1969). The same molting sequence for *O. virilis* was also described by Caldwell and Bovbjerg (1969) and Weagle and Ozburn (1972). As temperatures declined to below 10°C molting ceased, so no overwinter growth occurred. Temperatures below 15°C interfere with proecdysis in this species (Aiken 1969). For all age-groups in West Lost Lake and the Twin lakes fluctuations in densities were not high enough to elicit a predictable growth response. Changes in mean length between years usually varied only 1 or 2 mm at most in

TABLE 3. Summer population estimates of crayfish in three Pigeon River area lakes.

Lake and sex	Year	Age-group 0	I	II	III	Total
W. Lost						
♂	1966	24,809	12,022	13,150	521	50,502
	1967	18,584	1,823	3,009	70	23,486
	1968	8,641	2,887	1,799	47	13,374
	1969	42,983	3,350	2,109	33	48,475
♀	1966	30,187	6,713	3,758	34	40,692
	1967	28,412	2,137	1,453	113	32,115
	1968	12,436	2,545	1,313	28	16,322
	1969	21,171	2,109	2,178	34	25,492
N. Twin						
♂	1966	20,874	10,279	2,447	60	33,660
	1967	17,091	2,379	1,592	110	21,172
	1968	9,992	6,177	1,104	73	17,346
	1969	20,435	3,570	1,491	47	28,705
♀	1966	24,085	5,853	4,057	60	34,125
	1967	20,152	2,410	1,895	61	24,518
	1968	12,718	3,442	984	45	17,189
	1969	26,759	2,450	1,425	84	27,556
S. Twin						
♂	1966	19,290	6,606	5,314	—	31,210
	1967	15,905	2,324	1,670	91	19,990
	1968	9,936	15,893	1,270	—	27,099
	1969	16,048	3,329	1,692	16	21,085
♀	1966	20,296	3,119	1,621	—	25,036
	1967	20,340	1,745	1,448	32	23,565
	1968	24,824	6,852	1,454	50	33,180
	1969	16,048	1,797	1,301	—	19,076

carapace length and no consistent patterns were observed between density and mean size attained by an age-group in any given lake.

REPRODUCTION

Ovarian-egg counts varied annually in each lake (Table 5). Mean number of attached eggs also varied considerably from year to year and was usually much lower than mean ovarian counts (Table 5). The attached-egg counts were most representative of fecundity in these populations of crayfish (Table 5), since attached-egg stage is closer to hatching time. For *O. virilis* the disparity between ovarian- and attached-egg counts can be as much as 48% for any given year in the same population. Furthermore, the ovarian- and attached-egg counts approximated in only 1 case in 10 (Table 5). Spawning in the three lakes took place at approximately the same time, so the differences were not due to loss of eggs at differing periods of time.

In making comparisons between ovarian- and attached-egg counts, we were careful to adjust our raw data for attached-egg counts by examining only crayfish within the same length intervals, excluding individuals with undeveloped ovarian eggs which could not be accurately counted. The length frequencies of crayfish collected for ovarian- and attached-egg counts overlapped.

Population Dynamics

BIOMASS CHANGES

Fluctuations in mortality rates rather than growth rates produced most of the year-to-year differences in biomass accumulation. In general, the instantaneous rate of growth declined with age. It reached a maximum during the first summer of life, remained high during the second summer at age I, and then abruptly declined (Table 6).

Instantaneous mortality rates were quite variable compared to the growth rates. The highest rates were at age III (Table 6). The bulk of the annual production involved young-of-the-year and yearling age-groups. The instantaneous rate of growth (G) usually exceeded instantaneous mortality rate (Z), allowing a net increase in biomass during the second growing season.

At age II+ instantaneous mortality usually exceeded growth and biomass decreased (Table 6). Even though mortality rates reached their lowest levels at this age, growth rates decreased substantially, resulting in a much lower biomass for age II+ crayfish in most years.

During the fourth growing season (age III) instantaneous mortality rates were high in all the lakes, substantiating earlier findings in West Lost in 1962–63. A large die-off of age III crayfish always occurred between spring and midsummer. In some years males had the greater mortality but in general, no distinct pattern was discernible (Table 6).

Yearly changes in biomass between the lakes were the result of density-dependent control of mortality and fecundity. These factors helped regulate egg production and recruitment of the population and thus biomass change and annual production.

FECUNDITY CHANGES

Population density affected the reproductive capacity of crayfish. As the population in West Lost decreased sharply from 1966 to 1968 (Table 3), fecundity increased. During 1966–68 South and North Twin lakes had lower female breeding populations than West Lost and correspondingly

TABLE 4. Carapace length (mean ± SE), of crayfish from West Lost, North Twin, and South Twin lakes during late summer. Age-groups were determined by length-frequency analysis.

Year and age-group	Males No.	Males Length (mm)	Females No.	Females Length (mm)
		West Lost Lake		
1966				
0	79	13.8±0.12	221	13.4±0.11
I	437	26.9±0.09	1073	24.9±0.03
II	467	31.8±0.10	962	28.2±0.04
III	11	37.8±0.29	3	35.7±0.66
1967				
0	162	14.3±0.12	263	13.8±0.10
I	87	29.4±0.10	300	26.5±0.09
II	296	32.8±0.08	203	30.0±0.06
III	12	38.3±0.36	16	33.6±0.21
1968				
0	142	14.6±0.15	199	13.9±0.11
I	907	27.9±0.08	316	25.3±0.11
II	574	35.2±0.06	166	31.0±0.12
III	10	40.7±0.22	1	35.0±0.00
1969				
0	73	13.3±0.19	137	13.1±0.13
I	1423	29.3±0.05	400	25.6±0.07
II	895	34.9±0.07	413	29.4±0.07
III	13	41.1±0.08	6	36.3±0.18
		North Twin Lake		
1966				
0	130	16.3±0.14	150	15.5±0.14
I	503	29.6±0.07	197	29.9±0.07
II	75	34.6±0.19	5	35.4±0.51
III	2	40.5±0.50	—	—
1967				
0	134	15.3±0.16	158	14.5±0.14
I	429	31.3±0.08	334	26.6±0.08
II	288	34.4±0.07	262	30.4±0.09
III	19	38.4±0.22	8	36.5±0.42
1968				
0	130	13.4±0.12	160	12.9±0.11
I	1214	26.1±0.05	647	24.9±0.08
II	213	34.2±0.10	179	30.6±0.10
III	17	39.1±0.24	8	35.9±0.98
1969				
0	112	14.0±0.15	90	13.5±0.16
I	620	27.2±0.09	656	24.6±0.06
II	259	35.1±0.12	381	29.5±0.06
III	8	42.7±0.57	22	36.6±0.24
		South Twin Lake		
1966				
0	115	17.8±0.17	121	16.7±0.16
I	551	30.3±0.07	470	27.9±0.09
II	290	33.8±0.05	243	31.9±0.06
III	—	—	—	—
1967				
0	165	15.7±0.08	211	15.0±0.12
I	466	31.5±0.06	241	29.0±0.08
II	336	35.6±0.07	200	31.9±0.08
III	17	39.5±0.21	4	36.7±0.50
1968				
0	10	14.9±0.29	12	14.2±0.38
I	2067	26.6±0.07	1205	26.9±0.13
II	164	35.0±0.17	255	30.8±0.09
III	—	—	1	41.0±0.00
1969				
0	110	13.8±0.14	112	13.4±0.14
I	1535	32.5±0.03	292	27.1±0.10
II	779	35.9±0.04	212	30.5±0.10
III	5	41.6±0.12	—	—

TABLE 4. (*Concluded*)

a greater number of attached eggs per female (Table 5). The average number of attached eggs in the spring of a particular year in North and South Twin was negatively correlated with the increasing density of the adult female breeding population in the spring (Fig. 1).

The large decreases in the adult population are not the result of fish predation (Momot 1967b) or exploitation, since these lakes at this time were not open to crayfish harvest. The density changes are due to some other factor, perhaps food availability. A substantial number of dead unmolted adult crayfish were observed during the molting periods.

Whatever the exact cause of mortality, the main mechanism responding to population changes and controlling egg production seems to be egg resorption. This is indicated in comparing the increase in disparity between ovarian- and attached-egg counts with increases in population density.

In all three lakes as the population of age I+ and older crayfish decreased (Table 4), the difference between ovarian- and attached-egg counts decreased (Table 5). Thus, in West Lost, as the population decreased from 36,178 in 1966 to 8505 in 1967 the disparity between ovarian- and attached-egg counts was reduced from 28 to 0%. In 1968 the population numbered 8619 with a disparity of 26.7%. Similarly, in South Twin as the population decreased from 16,600 in 1966 to 7310 in 1967, the disparity correspondingly decreased from 19.5 to 6.8%. In 1968 the population rose to 25,469 and disparity increased to 40.3%. In North Twin the same trends are evident. As the population decreased from 22,756 in 1966 to 8447 in 1967 the disparity decreased from 48.5 to 16.7%. In 1968 the population reached 11,825 and the disparity climbed to 42.6%. We suggest that the differences may be due to a decrease in the amount of food per

TABLE 5. Disparity between the potential and actual reproductive capacity of crayfish in three lakes. Data presented for each egg count as $\bar{x} \pm$ SE (n), where n = number of crayfish examined.

Year	Length range of crayfish (mm)	Ovarian-egg count	Attached-egg count	Disparity % drop in egg count	Population density of age I+ crayfish
		West Lost Lake			
1966–67	22–32	93.1±5.1 (50)	66.8±5.5 (40)	28.3	36,178
1967–68	24–31	92.0±3.3 (52)	92.1±5.3 (45)	0.0[a]	8,505
1968–69	22–32	106.2±7.5 (30)	77.9±5.3 (49)	26.7	8,619
		North Twin Lake			
1966–67	23–32	136.3±9.9 (45)	70.4±5.1 (51)	48.5	22,756
1967–68	24–33	140.2±1.9 (34)	116.8±6.6 (33)	16.7	8,447
1968–69	22–35	143.5±8.7 (38)	82.1±6.3 (37)	42.6	11,825
		South Twin Lake			
1966–67	24–33	131.4±9.4 (24)	105.9±5.1 (47)	19.5	16,600
1967–68	26–33	155.9±6.6 (32)	145.3±5.4 (43)	6.8[b]	7,310
1968–69	22–33	132.5±6.8 (42)	80.4±5.2 (57)	40.3	25,469

[a]Not statistically significant; t .05 = 0.86, n = 97.
[b]Not statistically significant; t .05 = 0.109, n = 75.

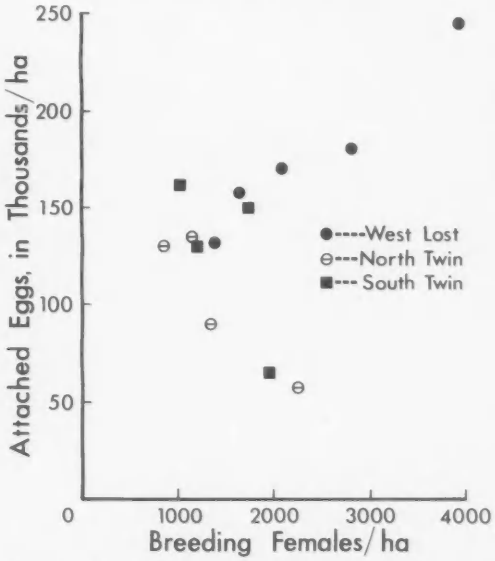

FIG. 1. Production of attached eggs on female crayfish in relation to density of breeding females in spring.

female adult with increasing density. This is then reflected in a decrease in the maturity of ovarian eggs resulting in the resorption of the eggs. Mason (1970) showed that the level of nourishment and degree of disturbance have a great deal to do with the propagative success of crayfish. Mason (1977a, b) showed that when crayfish were cultivated in laboratory tanks, grouped females had lower reproductive success than solitary females. He also showed that when incubated in the lab, 90% of the eggs stripped from females hatched and 76% of the eggs survived to the molt stage, when they normally become independent of the female. However, Aiken (personal communication) stated that in laboratory tanks crowded females bearing eggs would remove them over a period of days. The discrepancy between ovarian- and attached-egg counts is well known in the literature (Penn 1943; Smart 1962; Prins 1968; Payne 1971; Mason 1963; Goellner 1943). However, the exact importance of various mechanisms in accounting for such discrepancies has not been elucidated.

GROWTH

Growth did not respond in a predictable manner to changes in density. Growth was quite variable between years and between lakes in the same year (Table 4). The instantaneous rate of growth of the age 0 group during the first summer was found to vary inversely with the density of the population only in West Lost Lake. This is probably because of the higher density reached by age 0 crayfish in West Lost (Table 4).

Density had much less impact on the older age-groups in all the lakes. Their past growth history and relative density at earlier ages determined their rate of growth during the year in question. This was evident in age II and III groups, since most of the growth took place during ages 0 and I.

TABLE 6. Annual production of crayfish in three Pigeon River area lakes, 1966 and 1969. Z = instantaneous rate of mortality; G = instantaneous rate of growth. Totals are given in kilograms for each sex and combined sexes, and kilograms per hectare for combined sex in parentheses. W_0 = initial weight of stock; $\overline{W}$ = average weight of stock; $G\overline{W}$ = annual production.

Lake and sex	Age interval	Z	G	$G - Z$	W_0	$\overline{W}$	$G\overline{W}$	
				1966–67				
W. Lost								
♂	0.0–0.5	1.95	2.22	+0.27	11.2	12.9	28.6	
	0.5–1.5	2.61	2.56	−0.06	14.9	14.4	37.0	
	1.5–2.5	1.38	0.60	−0.78	70.2	48.7	29.6	
	2.5–3.5	5.24	0.55	−4.69	128.1	27.1	14.9	
								110.1
♀	0.0–0.5	1.74	2.22	+0.48	11.2	14.4	32.0	
	0.5–1.5	2.65	2.22	−0.43	18.1	14.7	32.7	
	1.5–2.5	1.53	0.54	−0.99	20.3	31.9	10.9	
	2.5–3.5	3.49	0.35	−1.14	25.4	4.6	1.6	
								77.2
						Total ♂ + ♀ (kg)		187.3
						(kg/ha)		(133.4)
N. Twin								
♂	0.0–0.5	0.98	2.72	+1.74	3.6	9.8	26.6	
	0.5–1.5	2.17	2.23	+0.06	20.6	21.2	47.3	
	1.5–2.5	1.87	0.45	−1.42	80.5	43.0	19.3	
	2.5–3.5	3.11	0.31	−2.80	30.8	10.3	3.2	
								96.4
♀	0.0–0.5	0.84	2.52	+1.68	3.6	9.4	23.8	
	0.5–1.5	2.31	1.91	−0.40	19.5	16.1	30.7	
	1.5–2.5	1.13	0.40	−0.73	33.1	23.5	9.4	
	2.5–3.5	4.20	0.56	−3.64	32.7	1.4	0.5	
								64.4
						Total ♂ + ♀ (kg)		160.8
						(kg/ha)		(84.6)
S. Twin								
♂	0.0–0.5	1.87	2.99	+1.12	3.3	10.7	31.9	
	0.5–1.5	2.12	1.98	−0.19	25.1	23.4	46.3	
	1.5–2.5	1.38	0.49	−0.89	55.5	36.7	18.0	
	2.5–3.5	4.07	0.46	−3.61	62.7	16.9	7.7	
								104.0
♀	0.0–0.5	1.62	2.87	+1.25	3.3	10.2	29.4	
	0.5–1.5	2.45	1.85	−0.55	23.3	17.5	32.5	
	1.5–2.5	0.54	0.41	−0.13	20.4	19.0	7.7	
	2.5–3.5	0.91	0.43	−0.48	15.9	12.6	5.5	
								75.0
						Total ♂ + ♀ (kg)		179.0
						(kg/ha)		(111.9)
				1967–68				
W. Lost								
♂	0.0–0.5	2.36	2.38	+0.02	6.1	6.1	14.5	
	0.5–1.5	1.86	2.31	+0.45	12.8	16.2	37.3	
	1.5–2.5	0.01	0.53	+0.52	14.2	18.1	9.6	
	2.5–3.5	4.16	0.66	−3.50	32.2	8.9	5.9	
								67.4
♀	0.0–0.5	2.01	2.22	+0.21	6.1	6.7	14.9	
	0.5–1.5	2.41	2.14	−0.27	16.2	14.2	30.4	
	1.5–2.5	0.49	0.50	−0.01	11.8	11.8	5.7	
	2.5–3.5	2.66	0.48	−1.18	11.8	6.9	3.3	
								54.3
						Total ♂ + ♀ (kg)		121.7
						(kg/ha)		(86.9)
N. Twin								
♂	0.0–0.5	2.57	2.34	−0.23	8.5	7.6	17.7	
	0.5–1.5	1.02	1.89	+0.87	13.5	21.5	40.7	

TABLE 6. (*Concluded*)

Lake and sex	Age interval	Z	G	$G - Z$	W_0	$\overline{W}$	$G\overline{W}$	
	1.5–2.5	0.77	0.21	−0.56	21.8	16.7	3.5	
	2.5–3.5	3.08	0.39	−2.69	19.6	6.8	2.6	
								64.5
♀	0.0–0.5	2.33	2.21	−0.12	8.5	8.0	17.7	
	0.5–1.5	1.77	1.81	−0.04	15.3	15.0	27.2	
	1.5–2.5	0.90	0.45	−0.45	13.3	10.8	4.8	
	2.5–3.5	3.24	0.51	−2.73	0.9	0.3	0.2	
								49.8
						Total ♂ + ♀ (kg)		114.3
						(kg/ha)		(60.2)
S. Twin								
♂	0.0–0.5	2.53	2.45	−0.08	8.5	8.1	20.0	
	0.5–1.5	0.01	1.92	+1.91	13.2	39.8	76.3	
	1.5–2.5	0.60	0.18	−0.42	22.0	18.0	3.2	
	2.5–3.5	—	—	—	23.0	—	—	
								99.5
♀	0.0–0.5	1.67	2.34	+0.74	8.5	12.6	29.4	
	0.5–1.5	0.41	2.05	+1.64	15.5	39.2	80.3	
	1.5–2.5	0.18	0.78	+0.60	12.8	17.6	13.7	
	2.5–3.5	3.37	0.78	−2.59	14.2	5.1	4.0	
								127.4
						Total ♂ + ♀ (kg)		226.9
						(kg/ha)		(141.8)
				1968–69				
W. Lost								
♂	0.0–0.5	1.01	2.22	+1.21	7.7	14.9	33.2	
	0.5–1.5	0.95	2.38	+1.43	6.0	13.4	32.0	
	1.5–2.5	0.31	0.67	+0.36	19.0	22.9	15.4	
	2.5–3.5	4.02	0.46	−3.56	23.9	6.5	3.0	
								83.5
♀	0.0–0.5	1.71	2.22	+0.51	7.7	10.0	22.2	
	0.5–1.5	1.77	2.12	+0.35	7.5	8.9	18.9	
	1.5–2.5	0.16	0.45	+0.29	12.3	14.3	6.4	
	2.5–3.5	3.65	0.48	−3.17	11.8	3.6	1.7	
								49.3
						Total ♂ + ♀ (kg)		132.8
						(kg/ha)		(94.8)
N. Twin								
♂	0.0–0.5	1.19	2.45	+1.25	4.4	8.8	21.5	
	0.5–1.5	1.03	2.18	+1.15	6.8	12.7	27.8	
	1.5–2.5	1.42	0.90	−0.52	32.9	25.7	23.1	
	2.5–3.5	3.17	0.67	−2.50	13.5	5.0	3.4	
								75.9
♀	0.0–0.5	0.92	2.34	+1.42	4.4	9.7	22.6	
	0.5–1.5	1.64	2.00	+0.36	7.6	9.2	18.4	
	1.5–2.5	0.88	0.50	−0.38	16.1	13.4	6.7	
	2.5–3.5	2.46	0.54	−1.92	8.5	3.8	2.0	
								49.8
						Total ♂ + ♀ (kg)		124.8
						(kg/ha)		(66.2)
S. Twin								
♂	0.0–0.5	2.00	2.45	+0.45	7.7	9.7	23.8	
	0.5–1.5	1.09	2.60	+1.51	7.5	17.6	45.9	
	1.5–2.5	2.25	0.91	−1.34	89.8	49.5	45.0	
	2.5–3.5	4.38	0.66	−3.72	14.5	3.8	2.5	
								117.2
♀	0.0–0.5	1.98	2.34	+0.36	7.7	9.3	21.7	
	0.5–1.5	2.63	2.16	−0.47	16.9	13.5	29.1	
	1.5–2.5	1.62	0.37	−1.25	40.5	23.1	8.5	
	2.5–3.5	—	—	—	24.3	—	—	59.3
						Total ♂ + ♀ (kg)		176.6
						(kg/ha)		(110.3)

TABLE 7. Effect of changes in density of the crayfish population on the annual mortality rate of adult and yearling crayfish.

Yearly interval	No. adults & yearlings per ha	Annual mortality rate			
		Adult		Yearling	
		♀	♂	♀	♂
		West Lost Lake			
1966–67	25,855	0.78	0.75	0.93	0.94
1967–68	6,146	0.39	0.01	0.91	0.84
1968–69	6,156	0.14	0.27	0.81	0.63
		North Twin Lake			
1966–67	11,976	0.68	0.84	0.90	0.89
1967–68	4,446	0.59	0.54	0.83	0.64
1968–69	6,224	0.59	0.75	0.60	0.69
		South Twin Lake			
1966–67	10,412	0.54	0.75	0.91	0.88
1967–68	4,569	0.17	0.45	0.66	0.00
1968–69	15,949	0.81	0.89	0.86	0.86

MORTALITY

Changes in mortality rates with fluctuations in density proved to be an important controlling factor for adults in all lakes and young-of-the-year in North and South Twin. In general, mortality was density dependent in male and female adult crayfish. The mortality of males increased faster than that of females (Table 7). For example, in West Lost Lake a fourfold decrease in density from 1966–67 to 1967–68 lowered the annual mortality rate of males from 0.75 to 0.01, while that of females decreased from 0.78 to 0.39. In North Twin Lake a threefold decrease in density between 1966–67 and 1967–68 lowered the annual mortality rate of males from 0.84 to 0.54 and of females from 0.68 to 0.59. In South Twin a twofold decrease in density between 1966–67 and 1967–68 lowered the annual mortality rate of adult males from 0.75 to 0.45 and adult females from 0.54 to 0.17. In yearlings, mortality showed little response to population change (Table 7). In addition, adult density had no apparent effect on the percent survival of age 0 crayfish from the time they are attached to the parent until the end of the first growing season.

RECRUITMENT

Since adequate recruitment is a key factor in sustaining a population, we measured the number of individuals added to the population during three life-cycle stages of a cohort. We first determined the potential recruitment possible in each lake. This was obtained by measuring the number of ovarian eggs produced by a female and multiplying this quantity by the size of the brood stock to give the ovarian-egg production. The realization of this potential egg production is expressed as the attached-egg production obtained by multiplying the mean attached-egg count by the number of female breeders in the population. These attached eggs soon hatch and must then survive to the end of the first growing season. We therefore determined the size of the year-class as age 0.5 crayfish at the end of the first growing season. Finally, any year-class must survive to reproductive age to make its maximum contribution to the population. If the population is under strong density control, the size of the brood stock should remain fairly constant despite fluctuations in the numbers of attached eggs and recruits of age 0 crayfish produced each season in the population.

Let us first consider potential egg production. Ovarian-egg production increased with the density of the female brood stock in all three lakes (Fig. 2). Despite high correlations the relationship was not statistically significant in West Lost and South Twin due to small sample size (North Twin: $r = 0.99$, $P < 0.01$; South Twin: $r = 0.94$, $P > 0.05$; West Lost: $r = 0.79$, $P > 0.05$). Still there is a high potential for the production of large year-classes in each lake as shown in Fig. 2. However this potential egg production is not realized in North Twin and South Twin lakes (Fig. 1). Negative correlations between the number of attached eggs per hectare and breeding females per hectare exist (North Twin: $r = -0.92$, $P > 0.05$, South Twin: $r = -0.612$, $P > 0.05$). These correlations were not statistically significant again because of small sample size but the trends are

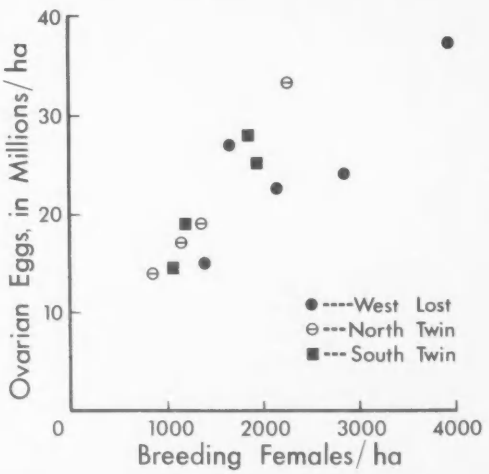

FIG. 2. Ovarian-egg production in relation to density of breeding female crayfish in spring.

quite indicative and opposite to the trends in West Lost.

In West Lost the reproductive potential is realized as the attached-egg production increased with the number of females in the population ($r = 0.97$, $P < 0.01$) (Fig. 1).

Of course these newly detached crayfish must of necessity survive to an age where reproduction can occur. Their survival was measured at the end of the first growing season by relating the number of young-of-the-year crayfish produced per adult female to the density of the female brood stock. There was no relationship in West Lost Lake ($r = -0.061$, $P > 0.05$). Negative correlations were observed in South Twin and North Twin lakes (South Twin: $r = -0.969$, $P < 0.05$; North Twin: $r = -0.633$, $P > 0.05$) (Fig. 3).

No relationship was shown between the size of the breeding population and the number of age 0 crayfish produced per hectare (North Twin: $r = 0.285$, $P > 0.05$; South Twin: $r = 0.226$, $P > 0.05$; West Lost: $r = 0.716$, $P > 0.05$) (Fig. 4).

Figure 4 results from combining the data of Fig. 1 and 3. Thus, the positive correlation in West Lost results from the positive and statistically significant relationship between the number of breeding females and attached-egg production (Fig. 1). In addition, in West Lost the percent survival of these attached eggs was high even when the number of hatchlings per hectare reached high densities. Despite a weak relationship between the survival and density of the young per hectare ($r = 0.202$, $P > 0.05$) (Fig. 5), in West Lost as the density of breeding

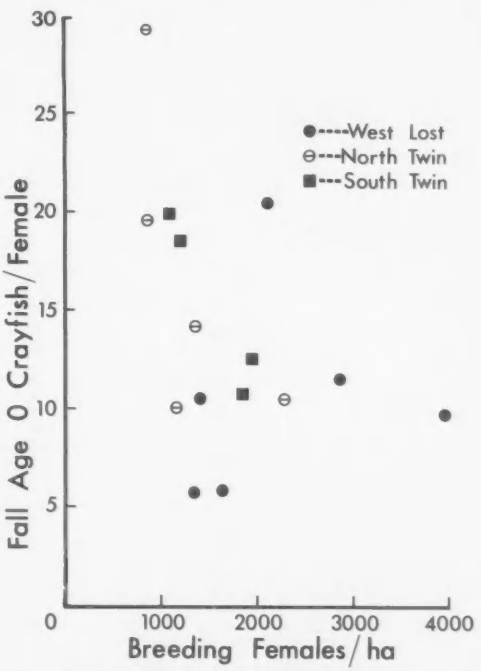

FIG. 3. Number of age 0 crayfish per breeding female in relation to density of breeding females in spring.

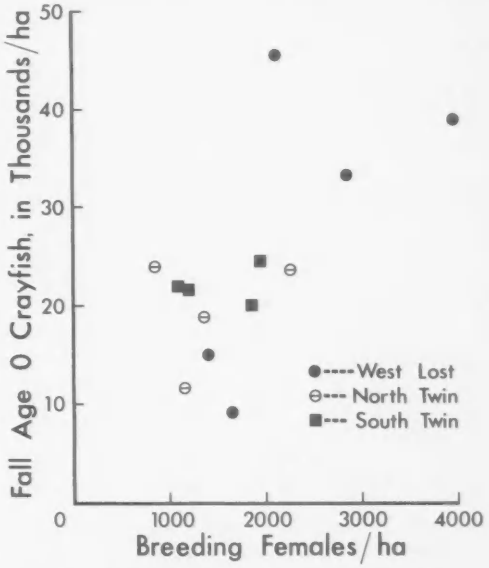

FIG. 4. Fall crop of age 0 crayfish in relation to spring density of breeding females.

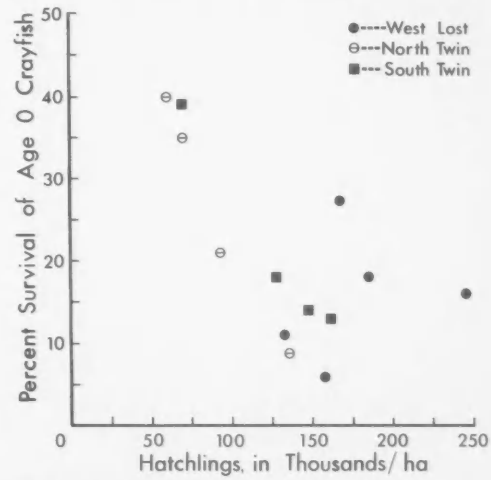

FIG. 5. Percentage survival of age 0 crayfish to end of first growing season in relation to initial density of hatchlings.

females increased, more eggs were produced and these had a greater chance of survival. Hence, substantially more young crayfish were produced in West Lost when the brood stock was large.

In contrast, no really large year-classes were produced in North and South Twin. In these lakes large numbers of attached eggs were produced at low densities (Fig. 1). However the survival rate of the young at these high densities is significantly reduced (North Twin: $r = -0.94$, $P < 0.05$; South Twin: $r = -0.97$, $P < 0.05$) (Fig. 5). As a result the number of age 0 crayfish is greatly reduced when the female breeding population increases and produces large numbers of hatchlings. Thus, recruitment of year-classes of age 0 crayfish is strongly regulated in North and South Twin but much less so in West Lost.

Despite differences in year-class production, the numbers of females surviving to reproductive age 2 yr later is strongly regulated in all three lakes. Strong population regulation resulted in two female breeding crayfish recruited for every two–six breeding females in the parental generation (Fig. 6). Thus, even in West Lost, where strong density-dependent controls do not operate on age 0 crayfish, the size of the brood stock as well as the fecundity of the brood stock is strongly regulated. Density-dependent mechanisms intervene between the time the young leave the female and become mature. The stronger controls on the crayfish populations of the Twin lakes are realized in examination of biomass accumulation in these three populations. Generally West Lost had larger maxima and greater variability in standing crops and annual production than the Twin lakes. This is shown in the next section.

FIG. 6. Density of age I and II brood stock females in relation to density of breeding females.

Annual Production and Turnover Ratio of Biomass

The biomass estimates for *O. virilis* in these pothole lakes were quite variable, ranging from 45.9 to 212.7 kg/ha (Table 8). In West Lost there was nearly a fourfold difference in biomass between the highest year (1966) and the lowest year (1968). In North Twin the difference was about threefold and in South Twin the difference was twofold (Table 8). West Lost Lake had the highest biomass recorded. This occurred in 1966. From that time on biomass continuously declined until 1968; a slight increase occurred in 1969. The pattern in North and South Twin was much the same (Table 8). The higher, more variable biomass in West Lost is the result of higher recruitment and less effective density controls on mortality of the younger age-groups described above. In general, the large populations reported for West Lost in 1962–63 were also found in other lakes in the area.

Similarly, annual production varied widely between lakes and within a lake from year to year. Once again, as expected West Lost had the greatest range of variation with annual production ranging from 86.9 to 133.4 kg/ha (Table 8). North Twin had the lowest annual production and the least variability, 60.2–84.6 kg/ha. The annual production in South Twin ranged from 110.3 to 141.8 kg/ha. Hence, the high production found in West Lost in 1962–63 extended to other lakes in the area. Despite quite variable biomass and production values the annual turnover ratio ($P:\bar{B}$) showed much less variation. For all three lakes the annual turnover ratio of the biomass (annual production divided by mean standing crop) varied from 0.94 to 1.53 but 9 of the 10 estimates were rather constant, between 0.94 and 1.35 (Table 8).

Population homeostasis seems characteristic of the crayfish populations in these lakes (Nicholson 1955, 1957). In general, the crayfish populations in South and North Twin appear to have strong density-dependent controls regulating fecundity, year-class recruitment, and density of the adult population, especially the female brood stock.

In West Lost strong density-dependent controls operate on fecundity and adult population but are less effective on the recruitment of year-classes. These differences in population dynamics are reflected in the annual production and biomass density of the crayfish in the lakes. In general, West Lost had greater variability and higher maxima than the Twin Lakes, especially North

TABLE 8. Estimated biomass ($\bar{B}$) in kilograms per hectare and annual turnover rato ($P:\bar{B}$) of crayfish during the summers of 1966, 1967, 1968, and 1969. P is annual production per hectare from Table 6; $\bar{B}$ is the mean annual biomass from 2 adjacent yr. $P:\bar{B}$ is then the ratio of the two values.

Year and sex	Age-group 0	I	II	III	Total 0–III	Total kg	kg/ha	$P:\bar{B}$
				West Lost Lake				
1966								
♂	14.9	70.2	128.1	8.6	221.8	297.8	212.7	
♀	18.1	32.0	25.4	0.5	76.0			
								0.94
1967								
♂	12.8	14.2	32.2	1.2	60.4	101.5	72.5	
♀	16.2	11.8	11.8	1.3	41.1			
								1.33
1968								
♂	6.1	19.1	23.9	1.0	50.1	82.1	58.6	
♀	7.5	12.3	11.8	0.4	32.0			
								1.32
1969								
♂	25.8	25.3	27.2	0.7	79.0	119.4	85.3	
♀	12.7	10.6	16.6	0.5	40.4			
				North Twin Lake				
1966								
♂	20.7	80.5	30.8	1.3	133.3	220.4	116.0	
♀	19.5	33.1	32.7	1.8	87.1			
								1.00
1967								
♂	13.5	21.8	19.6	1.9	56.8	102.2	53.8	
♀	15.3	13.3	15.9	0.9	45.4			
								1.19
1968								
♂	6.8	32.9	13.5	1.3	54.5	87.3	45.9	
♀	7.6	16.1	8.5	0.6	32.8			
								1.23
1969								
♂	15.5	21.5	19.5	1.1	57.6	98.9	52.1	
♀	18.2	10.9	11.0	1.2	41.3			
				South Twin Lake				
1966								
♂	25.1	55.5	62.7	—	143.3	202.9	126.8	
♀	23.3	20.4	15.9	—	59.6			
								1.17
1967								
♂	13.2	22.0	23.1	1.7	60.0	103.0	64.4	
♀	15.5	12.8	14.2	0.5	43.0			
								1.53
1968								
♂	7.6	89.8	14.5	—	111.9	193.7	121.1	
♀	16.9	40.5	24.4	—	81.8			
								1.19
1969								
♂	12.1	34.3	23.9	0.4	70.7	103.1	64.4	
♀	11.0	10.3	11.1	—	32.4			

Twin where density regulation seems the strongest. These populations quite possibly could be examined to test whether the stability of these populations is at the expense of the resilience of the populations (Holling 1973) in terms of their ability to respond to added stress.

The stability of the 10 independent estimates of the turnover ratio might have practical applica-

tion. If future studies prove other benthic species have stable turnover ratios, then confidence limits for such ratios can be calculated. Should the variation be shown to lie within statistically acceptable limits it might then be possible as a fish management tool to assign a food availability value to a lake in terms of its benthic production. This could then be based on the relatively easier task of identifying the major benthic species and measuring their standing crops. Estimated standing crops could be related to the annual production by multiplying the standing crop estimate by the annual turnover ratio. For any given year one could easily assess the potential annual production of each individual component and thus its potential significance as a food item for various fish. Kajak and Rybak (1966) showed that the profundal fauna in the waters they studied had a long developmental cycle and a turnover ratio between 2 and 6. The sublittoral fauna, with several generations per year, had a turnover ratio that ranged from 12 to 15. Most of the larger crustaceans have a turnover ratio between 0.9 and 2 (Cooper 1965; Andersson 1969). Mann (1969) cited two instances where attempts were made to infer production from biomass measurements. With the addition of more comparative data, this may become a real possibility in the future. Waters (1969) found that for a variety of benthic invertebrates the annual turnover ratio varied from 2 to 15, while the life-cycle turnover ratio varied from 2 to 5. The latter is obtained by taking the life of one cohort as the time interval during which biomass was elaborated and dividing by the mean biomass.

Our annual turnover ratios varied between 0.94 and 1.53, while the corresponding cohort turnover ratios varied between 5.12 and 5.94 (Momot and Gowing 1975). This is slightly higher than for most benthic insects just as Waters (1969) had predicted for most crustaceans.

Knowledge of the annual production of benthos in a lake would form a much sounder basis for the management of lakes for fish production than just a knowledge of standing crops of benthos. Standing crops do not measure food availability, since fish exhibit definite preferences and these change with the relative abundance of different food types (Ivlev 1961). In addition, the measurement of total average benthos per square metre may also be misleading, since not all species in the benthos are utilized by all kinds of fishes. The kind, as well as total amount, of benthos in a lake becomes an important characteristic of the suitability of a habitat as a producer of fish food.

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Results of an Experimental Fishery on the Crayfish *Orconectes virilis*

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MOMOT, W. T., AND H. GOWING. 1977. Results of an experimental fishery on the crayfish *Orconectes virilis*. J. Fish Res. Board Can. 34: 2056–2066.

Populations of the crayfish *Orconectes virilis* were exploited using the Paulik–Bayliff modification of the Ricker model in two small lakes. After harvesting with traps at maximum sustained yield (MSY) for 3 yr, fishing effort was increased fourfold in one lake, West Lost, for two successive seasons, while in North Twin Lake it was not changed. In West Lost yields were stable during MSY; yields increased with overexploitation, but growth and recruitment declined. Distortions occurred in the age composition of the catch. In North Twin this pattern was repeated except that in 1975 the catch dropped drastically even though fishing effort had not been increased. Because of declining recruitment, North Twin was actually overfished by about 20% in 1974 and greatly overfished in 1975. Decreased recruitment in both lakes can be attributed to two factors: (1) a decreased survival of hatchlings in both lakes due to a decline in nursery habitat; as a consequence the stock-recruitment relationship was altered; and (2) an increase in the density of age I (subadult) females due to the selective fishery for males; as a result density-dependent mechanisms lowered the survival of females from age I to II, producing fewer spawning adults. However, the high density of these age II females at age I decreased the egg production per adult female. The changes in the productivity of the lakes for crayfish during the period of exploitation were related to loss of microhabitat for hatchlings. This change in productivity offset the benefits of MSY management and resulted in a drastic drop in stocks when deliberate overfishing took place. West Lost gave good yields prior to its certain collapse. Yields in weight were an unreliable indicator of the impact of the fishery.

Key words: crayfish, *Orconectes virilis*, potential yield, production, recruitment, stock assessment, experimental fishery

MOMOT, W. T., AND H. GOWING. 1977. Results of an experimental fishery on the crayfish *Orconectes virilis*. J. Fish. Res. Board Can. 34: 2056–2066.

Les populations d'écrevisses *Orconectes virilis* de deux petits lacs ont été exploitées suivant la modification de Paulik–Bayliff du modèle de Ricker. Après avoir récolté les écrevisses à l'aide de casiers au niveau de rendement maximal soutenu (RMS) pendant 3 ans, on a quadruplé l'effort de pêche dans un lac, le lac West Lost, durant 2 saisons consécutives, alors qu'on l'a maintenu au même niveau dans le lac North Twin. Dans le lac West Lost, les rendements ont été stables durant la période de RMS; avec la surexploitation, les rendements augmentèrent, mais la croissance et le recrutement diminuèrent. Il se produisit des distortions dans la répartition des âges de la prise. Le phénomène se répéta dans le lac North Twin, sauf qu'en 1975, la prise diminua de façon dramatique, en dépit du fait que l'effort de pêche n'avait pas augmenté. Ceci est attribuable à un recrutement moindre. En fait, le lac North Twin a été surexploité d'environ 20% en 1974 et considérablement surexploité en 1975. La diminution du recrutement dans les deux lacs peut être attribuée à deux facteurs: (1) une diminution de l'habitat d'alevinage qui entraîna une diminution de la survie des larves nouvellement écloses dans les deux lacs. Ceci eut pour effet de modifier la relation stock-recrutement; (2) une augmentation de densité des femelles d'âge I (subadultes) à la suite d'une pêche sélective des mâles. Comme résultat, des mécanismes affectés par la densité abaissèrent la survie des femelles entre les âges I et II, produisant un nombre moindre d'adultes reproducteurs. Cependant, la forte densité de ces femelles d'âge II à l'âge I entraîna une diminution de production d'œufs par femelle adulte. On associe à la perte du microhabitat des larves les changements dans la productivité des lacs en écrevisses durant la période d'exploitation. Ce changement dans la productivité contrebalance les bienfaits de la gestion axée sur le

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RMS et entraîna une diminution dramatique des stocks quand on se livra à une surexploitation délibérée. Le lac West Lost donna de bons rendements avant un affaissement certain. Les rendements en poids ne furent pas un indicateur fiable de l'impact de la pêche.

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THIS study had two objectives. The primary objective was to determine the effects of exploitation on a population of freshwater crayfish. Literature on crustacean fisheries is sparse and one dealing with exploitation of freshwater crayfishes is virtually nonexistent in terms of modern fishery science (Miller and Van Hyning 1970; Threinen 1958). Our second objective was to evaluate maximum sustained yield (MSY) as a possible method for managing crayfish populations. Our experimental fishery provided an opportunity to test some of the hypotheses about a population response to the maximum yield harvest, namely that harvest of an unexploited stock produces the following changes: age composition would change, egg production should increase, growth should increase, recruitment should increase, and production should be higher (Ricker 1975). Recently, Larkin (1977) has advocated that the concept of MSY is not a viable management alternative because of unexpected biological complications leading to consequences that can flow from simple management strategies. Our study found that any strategy based on MSY must be compromised to reduce the risk of catostrophic decline.

The crayfish *Orconectes virilis* is abundant in our study lakes. Four years of data already existed on the dynamics of these exploited crayfish populations. We were therefore equipped to implement a maximum yield fishery and to precisely measure the effects of the fishery. We then deliberately subjected a crayfish population to overexploitation and monitored their response. We hoped to determine the mechanism by which crayfish adjusted to MSY exploitation and to levels of exploitation exceeding MSY. Further, we wished to see whether or not the population would collapse and if so, what evidence might forecast such an event.

Study Area and Methods

North Twin and West Lost lakes are located in the Pigeon River State Forest in the north central portion of the Lower Peninsula of Michigan, about 86 km south of the Straits of Mackinac. They are of similar geologic origin, size, morphometry, and general water chemistry (Tanner 1960; Momot and Gowing 1976, 1977), and as designated research waters, are closed to public fishing. The study was conducted from May 1970 to September 1975 and draws upon earlier work of 1966–69 (Momot and Gowing 1977).

Adult and yearling crayfish were collected with baited wire traps. Young-of-the-year were sampled with hand nets. In small lakes reliable estimates of crayfish population size can be made by the mark-and-recapture method during nonmolt periods. Crayfish can be given an easily recognized mark without evident effect on either growth or survival, by excision of one or more of their pleura (Goellner 1943; Momot 1967a, b). Population densities of crayfish were computed using the Schumacher–Eschmeyer formula. The population of adult females was estimated in the spring and summer, adult males in summer, and young-of-the-year in the summer. We successfully estimated population abundance in a crayfish population of known size with this technique (Momot 1967a). The accuracy of the estimates by the mark-and-recapture method was further corroborated by comparisons with (1) results derived by the Leslie method (Ricker 1975) and (2) ratios obtained from population estimates, compared to indices of abundance based on the catch per unit of trapping effort. The 95% confidence limits were usually within 10% of the estimate (Momot 1967a; Momot and Gowing 1976, 1977).

During their first growing season, young-of-the-year crayfish were found within and in the immediate proximity of beds of the sedge *Carex aquatilis* var. *substricta*. The beds occupied the immediate shoreline of the lake, usually to a depth 0.5 m, and formed a concentric band around the circumference of the lake. A vegetative index (D) to the quality of this emergent vegetation was determined for 1969 and 1973. This index (D) was constructed as follows: First we selected sample sites at 10-m intervals around the circumference of the lake. At each sample site we measured on a perpendicular line the linear width in metres of the vegetation band from the water's edge to the end of the vegetation. The clumps of vegetation within this linear distance were counted. A clump was counted if any portion of it touched the perpendicular line used to measure the width. The index was computed as the product of the width of the band times the number of clumps. The year 1969 was considered to be our base year for the period of nonexploitation and 1973 represented the exploitation years.

Age composition was analyzed from size-frequency polygons and verified by following modal progression of groups of previously marked animals of known age (Momot 1967a), as well as using arithmetic probability paper as described by Hopkins (1967). Carapace length was measured to the nearest 0.5 mm using vernier calipers. Growth rate and age were determined from a series of size-frequency graphs

plotted at intervals during the growing season (Momot and Gowing 1977).

Seasonal growth in weight was determined by converting length to weight using a length–weight relationship computed from empirical data. Instantaneous rates of natural mortality after sequential population estimates and instantaneous growth rates were used to estimate production (Ricker 1975). An example of the calculation of production is given in Momot and Gowing (1977).

Fecundity was determined from counts of eggs carried by females. Females were collected from May 15 to 25, approximately 2 wk before their eggs hatched. Recruitment was determined by population estimates made in the autumn for young-of-year crayfish hatched in the spring. (Momot and Gowing 1977). In the spring recruitment is given as numbers of hatchlings per square metre. This represents the number of attached eggs present per square metre littoral zone. In the autumn recruitment is measured as the number of young-of-the-year per square metre littoral zone. We created a fishery in 1970 subject to regulations based on our knowledge of crayfish population dynamics. In 1970, 30 baited traps were fished continuously for 5 consecutive days during the last week of August for a total of 150 trap days in North Twin and West Lost lakes. Because females bred once before reaching 30 mm and were correspondingly older than males of the same size, only crayfish >30 mm carapace length were removed. Mainly age II and III males were harvested from the population due to the selectivity of this gear towards males at this time of year. After an initial harvest, the application of the Paulik–Bayliff (1967) modification of the Ricker equilibrium yield model indicated a maximum yield was achieved in West Lost but that the population in North Twin was being underharvested. The model prescribed a doubling of effort to at least 300 trap days for North Twin. Therefore, during 1972 and 1973 we used 60 traps for a 5-day period in North Twin. During 1974 and 1975 we increased the effort in West Lost Lake fourfold to 600 trap days (120 traps for 5 days), while in North Twin Lake we retained the 300 trap-day effort.

Results

Yield

Males dominated the catch by the fishery in both lakes (Table 1). The fishery in West Lost and North Twin was maintained at a maximum level from 1970 to 1973. In West Lost Lake the highest yield (25 kg/ha) was attained during the 1st yr (1970) of the fishery. Thereafter the yield remained quite stable (8.9–11.5 kg/ha) until trapping effort was increased in 1974 (Table 1). Thus, it followed almost a classical pattern in its development (Ricker 1975) whereby a high initial yield is followed by a stable yield during MSY management. North Twin Lake did not show the initial large yield but remained at a stable level throughout the 1970–73 period of

TABLE 1. Yield of male and female crayfish, 30-mm carapace length and larger, in West Lost and North Twin lakes. In West Lost 150 trap days were employed annually from 1970 to 1973 and 600 trap days in 1974 and 1975; in North Twin 150 trap days in 1970 and 1971 and 300 trap days annually from 1972 to 1975. Total yield in kilograms per hectare given in parentheses. A = available; H = harvested.

Sex and age-group	West Lost Lake			North Twin Lake		
	Number			Number		
	A	H	%	A	H	%
			1970			
Male						
I	2459	656	26	1756	68	4
II	2576	2305	89	1369	775	57
III	36	34	94	16	17	100
Total	5071	2995	58	3141	860	27
Female	3989	15	<1	2401	45	2
Total catch		3010 (25.0)			905 (5.7)	
			1971			
Male						
I	1694	309	18	1611	183	11
II	1588	1103	69	1113	676	61
III	34	19	56	40	34	85
Total	3316	1431	42	2764	893	32
Female	4945	106	2	4495	30	<1
Total catch		1537 (11.5)			923 (5.3)	
			1972			
Male						
I	1688	168	11	1462	406	28
II	1442	931	65	1576	898	57
III	183	125	68	41	13	32
Total	3313	1244	38	3079	1317	43
Female	6865	5	<1	5440	96	2
Total catch		1249 (10.3)			1413 (8.7)	
			1973			
Male						
I	1492	370	19	1915	115	6
II	1294	741	57	1293	641	50
III	70	28	40	185	79	43
Total	3306	1139	34	3393	835	25
Female	3407	72	2	4697	59	1
Total catch		1211 (8.9)			894 (5.2)	
			1974			
Male						
I	3590	1444	40	2806	1419	51
II	1686	629	37	1053	836	79
III	80	20	25	28	32	100
Total	5356	2093	39	3887	2287	59
Female	1059	29	3	1322	207	16
Total catch		2122 (17.0)			2494 (14.2)	

TABLE 1. (*Concluded*)

Sex and age-group	West Lost Lake			North Twin Lake		
	Number			Number		
	A	H	%	A	H	%
			1975			
Male						
I	1779	0	0	2899	68	2
II	2415	1721	71	438	313	72
III	42	7	17	11	11	100
Total	4236	1728	41	3348	392	12
Female	897	54	6	446	64	14
Total catch		1782 (13.1)			456 (2.6)	

the fishery for a maximum sustained yield of 5.2–8.7 kg/ha.

CHANGES IN AGE COMPOSITION

According to theory, as an unexploited population is first cropped the percentage of young animals in the population should increase (Ricker 1975). So we initially expected a change in population structure with fishing, but these changes, if present during the period of maximum sustained yield were obscured by variability in cohort mortality rates. This was also the case for females subject to low fishing mortality because of their avoidance of traps in late summer. Momot and Gowing (1972) showed that adult females migrate to deep, cold waters and do not enter traps as readily as males, which are present in shallow, warm waters. Male dominance excluding females from these shallow waters is probably responsible for their movement to deeper, colder waters.

Cohort growth rates largely determined availability and yield throughout the course of the fishery. In West Lost Lake the fourfold increase in effort in 1974 and 1975 increased the percentage of the total male population cropped only slightly over that in 1972 and 1973 and failed to match the first 2 yr of the fishery (Table 1).

With overexploitation in West Lost Lake in 1974 and 1975, the age composition of the harvests changed. In both years the 1973 cohort made up the bulk of the catches. Thus, in 1975 the fishery sustained a high yield by intensive exploitation (71.3%) of age II crayfish (Table 1). Because both of the 1974 and 1975 year-classes were of average size only, continued intensive exploitation would have caused a drop in 1976 catches (Table 1).

In 1974 and 1975 North Twin Lake was fished to provide the maximum sustained yield as determined in 1971 by the Paulik–Bayliff program. The catch more than doubled in 1974 but decreased sharply in 1975. In contrast to the years 1970–73, large numbers of age I crayfish appeared in the catch of 1974. These crayfish were 29.9 mm in mean length, large enough to enter the fishery in substantial numbers and to greatly increase the catch. However age I crayfish in 1974, because of high natural mortality, had poor survival to age II in 1975, which subsequently reduced the yield drastically. Whether the yield would have stabilized or diminished in 1976 probably depended on the survival of the 1975 year-class (age 0) which was below average density. Thus, unusually rapid growth of age I crayfish in 1974 led to average yields, masking the effects of lowered recruitment and overexploitation.

FECUNDITY

As an unexploited population is cropped, older individuals should be replaced by younger, more efficient crayfish due to lessening of intraspecific interactions which, in turn, could bring about increased egg production (Ricker 1975). However, the mean number of attached eggs diminished steadily in both lakes until 1975. The decrease was probably due to a high density of age I crayfish. Crowding adversely affected fecundity in both lakes (Momot and Gowing 1977). In 1974 the number of age I female crayfish was considerably reduced, resulting in a compensatory increase in fecundity in 1975 (Table 2).

There was an inverse relationship between density of adult females and number of attached eggs per adult female (Fig. 1). (Due to small sample size none of the correlations are significant: West Lost, preexploitation $r = 0.66$, $t = 1.52$, exploitation $r = 0.61$, $t = 1.65$; North Twin, preexploitation $r = 0.54$, $t = 1.11$, exploitation $r = 0.43$, $t = 0.83$.) Since exploitation began, the numbers of attached eggs per female adult have been considerably below those of the preexploitation period (Fig. 1). The decrease has not been the result of a change in the average size of the females. The mean size of females in West Lost Lake was 29.5 mm before exploitation and 28.6 mm during exploitation, while in North Twin mean size was 31.1 before and 29.5 during exploitation. The mean size of adult female crayfish from 1966 to 1970 and from 1971 to 1975 was not significantly different in either lake (West Lost: $t = 1.638$, $P > 0.05$; North Twin: $t = 1.391$, $P > 0.05$). Differences are probably due to competition for food, which could control egg production. As density of females increased, the percent disparity between ovarian-egg counts and

TABLE 2. The mean number of attached eggs per female crayfish in West Lost and North Twin lakes from 1966 to 1975, determined at approximately two weeks before hatching.

Lake and year	No. of female crayfish examined	Egg counts (mean ± SE)
West Lost		
1966	73	61.9±3.8
1967	42	64.8±5.6
1968	43	92.1±5.3
1969	52	78.4±5.5
1970	51	64.8±5.8
1971	62	39.4±4.8
1972	51	52.5±6.4
1973	42	44.4±5.7
1974	38	27.4±3.7
1975	30	53.5±6.4
North Twin		
1966	78	25.7±3.3
1967	54	68.0±5.5
1968	33	116.8±6.6
1969	37	82.1±8.3
1970	52	51.3±4.9
1971	33	56.2±7.2
1972	54	53.4±5.7
1973	54	42.4±4.9
1974	47	23.4±2.6
1975	30	86.2±11.6

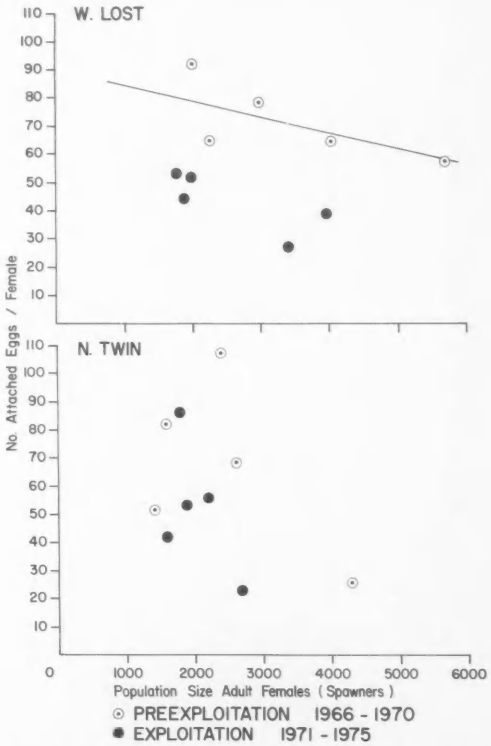

FIG. 1. Number of attached eggs per female adult crayfish as related to size of spawning female population in West Lost Lake (*upper*) and North Twin Lake (*lower*).

attached-egg counts increased (Momot and Gowing 1977). With increased density, the amount of food available per female is decreased. Since female adults are confined to deeper, cooler waters, the amount of food present in these deeper waters could serve to limit female population size.

GROWTH

As older members of an unexploited population are first cropped, the reduction in intraspecific interaction should result in an increased growth of young animals (Ricker 1975). This did not happen. Growth rates of all age-classes have been variable since 1966. Excluding 1975, the instantaneous rate of growth of age I male crayfish increased with fishing. There was no evident relationship between the instantaneous rate of growth of age II and III crayfish of both sexes and population density except for the very evident low rates in North Twin in 1975 (Table 3).

RECRUITMENT

One of the anticipated responses of an unexploited population to fishing is an increase in recruitment due to a higher reproductive efficiency of younger age-groups (Ricker 1975). Recruitment of age 0 crayfish at the end of their first growing season was reduced in both lakes throughout the period of exploitation from 1971 to 1975 (Fig. 2). This occurred because of a lower fecundity (Table 2) and small brood stocks (Table 4). Despite the large numbers of age I females generally recruited annually, poor survivorship from age I to II resulted in relatively small breeding stocks of age II females (Table 4). Also, fecundity may have been suppressed because of high densities during sexual maturation the preceding fall and summer (Table 4) (Momot and Gowing 1977). In 1974 a small population of age I crayfish in each lake was the progenitor of a small brood stock in 1975. However, a large number of young hatched in 1975 produced few young recruits per square metre of littoral area (Fig. 2).

Abundance of parental stock was compared with that of its progeny using Ricker's reproduction curves (Ricker 1975). Data prior to the

TABLE 3. Instantaneous growth rates (G) of crayfish from West Lost and North Twin lakes from 1966 to 1975.

Sex and age group	1966–67	1967–68	1968–69	1969–70	1970–71	1971–72	1972–73	1973–74	1974–75
					West Lost				
Male									
0	2.22	2.38	2.22	2.22	1.62	2.22	2.11	2.03	2.18
I	2.56	2.31	2.38	2.34	2.34	2.86	2.41	2.49	2.39
II	0.60	0.53	0.67	0.48	0.58	0.57	0.71	0.71	0.27
III	0.55	0.66	0.46	0.46	0.45	0.41	0.58	0.69	0.32
Female									
0	2.22	2.22	2.22	2.22	1.52	2.22	2.03	1.93	2.09
I	2.22	2.14	2.12	2.03	2.10	2.78	1.99	2.26	2.23
II	0.54	0.50	0.45	0.35	0.47	0.36	0.30	0.51	0.37
III	0.35	0.48	0.48	0.68	0.36	0.37	0.41	0.62	0.45
					North Twin				
Male									
0	2.72	2.34	2.45	2.22	1.86	2.20	2.22	2.28	2.09
I	2.23	1.89	2.18	1.83	2.29	2.78	2.25	2.48	1.97
II	0.45	0.22	0.90	0.63	0.85	0.77	0.50	0.90	0.44
III	0.31	0.40	0.67	0.56	0.48	0.62	0.28	0.64	0.37
Female									
0	2.52	2.22	2.34	2.13	1.86	2.16	2.22	2.22	2.11
I	1.91	1.81	2.00	1.83	2.19	2.52	2.21	2.25	1.82
II	0.40	0.45	0.50	0.58	0.53	0.51	0.51	0.57	0.20
III	0.56	0.52	0.54	0.68	0.33	0.48	0.36	0.40	0.24

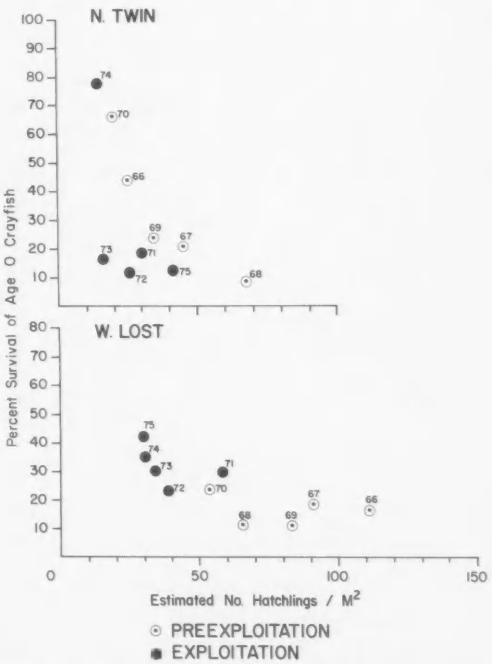

FIG. 2. Percent survival of age 0 crayfish as a function of their estimated density at hatching in North Twin Lake (*upper*) and West Lost Lake (*lower*).

fishery suggested that West Lost Lake produced more age 0 recruits than North Twin Lake (Fig. 3). The greater relative abundance of the sedge *Carex aquatilis* var. *substricta* in West Lost Lake accounted for the higher production of young (Table 5, Fig. 3). The *Carex* band serves as a nursery area for the young-of-the-year, providing shelter from predators, especially dragonfly nymphs, and food for the young from the time they leave the female until they are about 20 mm in cephalothorax length (Dye and Jones 1975).

During the period of exploitation (1971–75) the water level in the two lakes increased greatly, exceeding levels of 1966–70. Much of the emergent vegetation was inundated and index values (D) in the two lakes declined (Table 5). Higher water levels affected both density and linear extent of the vegetation. Whereas in 1969 West Lost Lake had a much higher D value, by 1973 it was nearly the same as in North Twin Lake (Table 5). However, the density of the vegetation in West Lost was always greater. The mean number of clumps per metre of width averaged 4.8 in West Lost, but only 2.4 in North Twin. Consequently, while the quality of the microhabitat declined in both lakes, it still was considerably better in West Lost than in North Twin (Table 5).

We also measured the influence of physical factors, notably temperature and mean monthly precipitation, on recruitment. No correlation was

TABLE 4. The number of subadult female crayfish (age I) present in the summer compared at maturity (age II) the following spring.

Lake and year	No. age I females present in summer	No. age II females present the following spring
West Lost		
1970	3387	2900
1971	2893	1710
1972	5107	1801
1973	2144	1936
1974	1203	1027
1975	1526	
North Twin		
1970	1440	1365
1971	3039	1504
1972	3835	1432
1973	3607	2202
1974	1218	1047
1975	1789	

observed among these variables. We also examined the relationship of lake water level to three aspects of recruitment, as follows: (1) egg production per square metre of littoral area, (2) number of recruits per square metre of littoral area, and (3) percentage survival from hatching in spring until fall. Water level did not directly affect recruitment of year-classes in the lakes, but could do so indirectly. Abrupt changes in water level did alter microhabitat (*Carex* beds) both in quantity and quality. Since the microhabitat serves as a nursery ground for the young, the effect of the exploitation for a maximum sustained yield, which brought into play a self-regulatory mechanism in the relationship between female density and fecundity (Fig. 1), was confounded by the effect of habitat loss on the subsequent survival of the young (Fig. 3).

Thus, the sex-selective fishery for males increased the size of the female population at age I by reducing competition between the males and females. The crowding of females at age I reduced fecundity at age II. In addition, survival of age I females to age II was poor. Recruitment was reduced because fewer newly hatched young were produced. Concurrent loss of the nursery area further reduced the survival of these newly hatched young. As a result of these events recruitment declined at a steady rate and when the stock was overfished, collapse was very rapid.

ANNUAL PRODUCTION, MEAN BIOMASS, AND TURNOVER RATIO

Production and biomass decreased in both lakes during the years of exploitation, reaching their lowest levels in 1975 (Table 6). Differences in mean annual production between 1966–70 and 1971–75 were significant in both lakes (West Lost: $t = 3.333$, $P < 0.05$, North Twin: $t = 3.049$, $P < 0.05$). Differences in mean biomass between these two periods were significant at the 0.05 level in North Twin ($t = 3.378$), but only at the 0.10 level in West Lost ($t = 2.174$). Differences in the $P:\overline{B}$ ratio were not significant. However, during the period of overharvest (1974–75) the $P:\overline{B}$ was 0.75 in West Lost compared to a mean of 1.21 for the previous years. In North Twin the $P:\overline{B}$ in 1974–75 was 0.54 compared to an average of 1.11 for the previous years (Table 6). A significant decline in annual $P:\overline{B}$ ratio could indicate overexploitation. More data are needed to verify this observation.

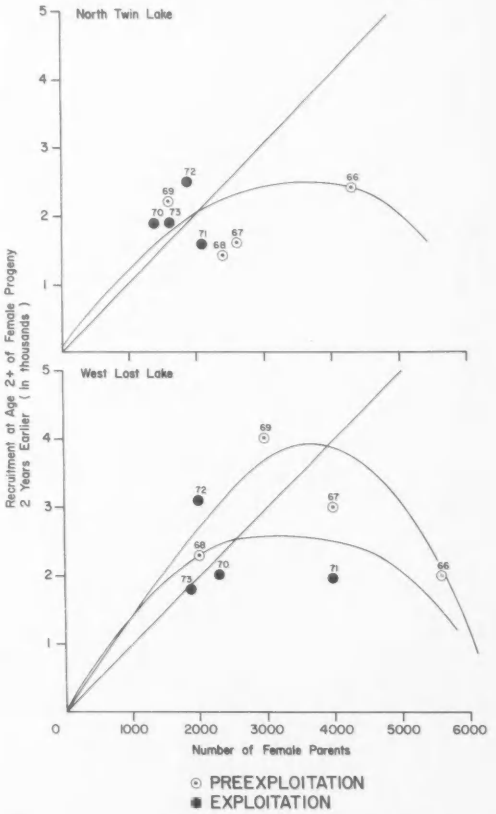

FIG. 3. Stock–recruitment curve for North Twin Lake females (*upper*) and West Lost Lake females (*lower*).

Data on cohort production, biomass, and turnover ratios are available (Momot and Gowing 1975, 1976), but they revealed few specific

TABLE 5. Vegetative index (D) of the available microhabitat of young-of-the-year crayfish.

Year	(A) No. samples	(B) No. clumps per interval (mean ± SE)	(C) Linear distance (m) of vegetation band (mean ± SE)	(B × C) D	(B/C) Density
			West Lost		
1969	44	5.07 ± 0.50	0.976 ± 0.098	4.94	5.19
1973	42	2.14 ± 0.24	0.446 ± 0.045	0.95	4.79
			North Twin		
1969	54	3.65 ± 0.40	0.601 ± 0.690	2.19	6.07
1973	54	1.48 ± 0.23	0.616 ± 0.630	0.91	2.40

TABLE 6. Mean summer biomass ($\bar{B}$), production (P), and the annual turnover ratio (P:$\bar{B}$) of crayfish in West Lost and North Twin lakes.

Lake and year	$\bar{B}$	P	P:$\bar{B}$
West Lost			
1966–67	142.9	133.8	0.94
1967–68	65.6	86.9	1.33
1968–69	71.9	94.9	1.32
1969–70	74.8	91.0	1.22
1970–71	58.7	61.3	1.05
1971–72	55.6	76.6	1.38
1972–73	51.7	56.6	1.10
1973–74	52.2	70.3	1.35
1974–75	51.8	38.7	0.75
North Twin			
1966–67	82.4	84.6	1.02
1967–68	49.9	60.2	1.20
1968–69	78.2	65.7	0.85
1969–70	44.1	47.8	1.08
1970–71	33.0	45.9	1.39
1971–72	33.9	43.7	1.28
1972–73	35.6	33.6	0.94
1973–74	38.2	44.0	1.15
1974–75	31.7	17.0	0.54

trends. Although cohort production was significantly lower during exploitation in North Twin ($t = 3.964$, $P < 0.05$), none of the other comparisons proved significant. Because crayfish have a 3-yr generation cycle, overfishing should be extended for at least this long to provide clear-cut results.

Discussion

Effect of Maximum Sustained Yield on Crayfish Population

One of our primary objectives was to test some hypotheses about maximum sustained yield, measuring benefits of such a strategy for the crayfish population and the fishery. One of the ecological tenets of MSY management is that it should maximize production. Increased yields from this higher production would benefit the fishery. Our study showed that changes in the productivity of the experimental lakes due to loss of nursery area severely modified results expected from the scheme for a maximum sustained yield. Such changes would be expected to modify and perhaps negate any application of the concept of a maximum sustained yield to other fisheries and should be a problem of great concern to fishery managers (Larkin 1977).

As anticipated, our fishery produced a sustained yield in number and weight of crayfish. The low yield in North Twin in 1973 was caused by a lower efficiency in harvest rather than a decrease in population size (Table 1). However, fishing at the level needed for a maximum sustained yield did not lead to some of the predicted biotic responses. No great shift in age composition toward greater numbers of young animals was noted during the period of maximum sustained yield. In fact, age I males were less abundant than before exploitation occurred. This decrease, however, was accompanied by an increase in the numbers of age I females (Table 4). In turn, the survival of these females to age II decreased, as did fecundity caused by crowding at the sublegal level. The resulting egg production and eventual recruitment did not respond to exploitation as expected. Presumably this response might not have occurred had both sexes been available for harvest. At the same time, the available habitat decreased and presumably reduced recruitment, resulting in lower mean summer biomass and annual production. The decrease in production among males in West Lost involved primarily age 0, I, and II animals (Momot and Gowing 1976). In North Twin Lake the greatest decrease was among age 0 crayfish. However, these reductions were already evident in 1970–71 and affected primarily age-groups not subject to harvest. The decrease was caused by poor recruitment, not by excessive removal in the fishery. Cohort production supported this conclusion. The 1970, 1971, and 1972 cohorts were thus produced since exploitation began, and they show a lower production value for age 0 and I males than for earlier cohorts. This was caused mainly by poor recruitment, since growth had not declined and instantaneous growth rates had not changed (Table 3). No evident trends or changes were noted for the instantaneous mortality rates for these same age-groups during the period of sustained yield (Momot and Gowing 1976).

The fishery, by protecting females, had no direct effect on recruitment. The readjustment in

TABLE 7. Equilibrium yield matrices for male crayfish in West Lost Lake from 1970 to 1972 and North Twin Lake from 1970 to 1975. Age at entry is 2.5. In West Lost Lake the unit of effort of 1X was 150 trap days from 1970 to 1972. In North Twin the unit of effort was 1X in 1970 and 2X in 1971; and 1X in 1972–75, equivalent to 300 trap days. Asterisk denotes rate at which the fishery operated.

Lake, year, effort, and age at entry	Yield					
	Kilograms per 1.0 kg recruits					
West Lost Lake						
1970–71						
Effort	0.50X	0.75X	1.00X*	1.25X	2.00X	
1.5	1.54	1.53	1.51	1.49	1.43	
2.5	1.58	1.60	1.60	1.60	1.60	
3.5	1.42	1.52	1.56	1.57	1.59	
1971–72						
Effort	0.50X	0.75X	1.00X*	1.25X	1.50X	
1.5	1.26	1.45	1.55	1.59	1.61	
2.5	1.26	1.47	1.58	1.65	1.69	
3.5	0.64	0.81	0.94	1.03	1.11	
North Twin Lake						
1970–71						
Effort	0.75X	1.00X*	2.00X	3.00X	4.00X	
1.5	1.02	1.09	1.27	1.34	1.40	
2.5	1.01	1.09	1.27	1.35	1.43	
3.5	0.58	0.59	0.59	0.59	0.59	
	Kilograms per 1.0 kg recruits					
1971–72						
Effort	1.25X	1.50X	2.00X*	3.00X	4.00X	
1.5	1.75	1.80	1.87	1.94	1.97	
2.5	1.76	1.81	1.89	1.97	2.01	
3.5	1.04	1.06	1.09	1.112	1.13	
	Kilograms per 5.49 kg recruits					
1972–73						
Effort	0.50X	0.75X	1.00X*	1.50X		
1.5	5.36	7.51	10.62	8.65		
2.5	0.40	0.55	1.24	1.32		
3.5	1.76	2.46	9.96	8.57		
	Kilograms per 2.80 kg recruits					
1973–74						
Effort	0.25X	0.50X	0.75X	1.00X*	2.00X	
1.5	0.26	0.51	0.76	0.98	1.82	
2.5	0.64	1.26	1.70	2.13	3.53	
3.5	0.80	1.42	1.89	2.04	2.46	
	Kilograms per 4.30 kg recruits					
1974–75						
Effort	0.50X	0.65X	0.75X	0.80X	0.85X	1.00X*
1.5	4.80	5.72	6.25	6.44	6.66	7.19
2.5	2.51	2.83	3.01	2.97	3.04	2.64
3.5	2.78	2.92	3.00	3.00	2.70	0.98
	Kilograms per 4.73 kg recruits					
1975–76						
Effort	0.75X	0.90X	1.00X*	2.00X		
1.5	0.20	0.23	0.25	0.46		
2.5	2.04	2.23	2.34	2.86		
3.5	0.97	0.98	0.98	0.81		

population densities between males and females, combined with environmental changes, caused decreases in fecundity and recruitment. Exploitation did not decrease production and harvest but neither was there an increase in these parameters, as had been expected.

Effect of Overfishing

In West Lost Lake yield was increased by overfishing in 1974 and 1975, with the yield dropping in 1975, though still above average. In spite of these yields, evidence of overfishing became noticeable when the age composition of the catch was examined. The increased stress on the population suppressed the normal growth response and so this resulted in decreased growth rates reflected in the mean size of individuals at a given age and the poor production of recent cohorts.

In North Twin Lake we retained the 300 trap-day effort through 1974 and 1975. Because of failing recruitment, this resulted in a 20% overharvest in 1974 and a drastic overharvest in 1975. Overharvest in North Twin is seen in a comparison of yield matrices from 1974 and 1975 with earlier years (Table 7). Growth and mortality rates for 1975 were used to generate a yield matrix for the 1975 fishing rate (Table 7). At all multiples of the 1975 fishing effort, the yields are below the replacement level of recruitment. The yield increased in 1974 due to a large catch of age I crayfish (Table 1). The yield in 1975 was very low. The slow growth of age I males made most of them unavailable to the fishery, while the number of age II males was greatly reduced due to poor survival from the year before. The catch in both lakes would have been much lower in 1974 if it had not been for the rapid growth of age I crayfish. Besides changes in age composition of the catch, the population in North Twin also had poor growth and recruitment.

Rate of growth during the sustained level of exploitation largely determined yields to the fishery. Length of the growing season determined to a great extent the size of the harvestable stock during overfishing. Declining growth rates in 1975 distorted age composition of the catch and reduced the size of the harvestable stock. The reduced recruitment that accompanied overfishing resulted mainly from a shift in the relationship between egg production and female density brought about by the confounding effects of a shrinking nursery area previously discussed. Changing egg production and decreasing recruitment combined to lower the stock–recruitment curve.

Fluctuation in yield from year to year despite the stability of the population may be peculiar to a crustacean trap fishery. The present study suggests that the use of catch per unit effort and age composition data by themselves to model the effects of harvest on a crayfish population is hazardous. Yields in weight were an unrealiable indicator of the impact of the fishery on the stock. The West Lost Lake stock gave good yields immediately prior to its probable collapse. Changes in annual $P:\overline{B}$ ratio might serve as a better indicator of imminent collapse.

Effect of Maximum Sustained Yield on Fishery

Benefits of this strategy for management of crayfish stocks can be drawn from some comparisons between our fishery and one for this species in Wisconsin. In Wisconsin typical commercial catches averaged 7.2–7.5 crayfish per trap (Threinen 1958). In West Lost Lake the initial catch per unit of effort exceeded the 7.2 mean but fell below this level by the 5th day of harvest. North Twin Lake provided a marginal crayfish fishery. At twice the effort the total catch increased, but the catch per unit effort fell considerably below the 7.2 level. The Wisconsin lakes are considerably larger in area than our experimental lakes. One of the best fishery sites in Wisconsin was estimated to have produced 37.3 kg/ha. Our best yield was 24.9 kg/ha in West Lost Lake in 1970. However, from 1970 to 1973 the yield averaged about 14.3 kg/ha. Low standing crops preclude an economically viable fishery in lakes of the Pigeon River area. At the intensity with which we fished, we cropped 25–60% of the available biomass within the 5-day period. Many lakes have standing crops much greater than those found in our lakes (Momot et al. 1977). Under intensive cultivation, yields approaching 500 kg/ha are not unusual, and wild crayfish stocks often exceed such yields (Momot et al. 1977).

The harvest of wild stocks of crayfish can be managed for high yields. For *O. virilis* present methods of harvest do not affect females, so our maximum-yield strategy afforded maximum protection to the spawning stock. In this case, even if size limits had not protected females, their low susceptibility to traps would have been more than adequate. In addition, the maximum protection in these lakes was achieved at age 1.5 and at a length of 30 mm. With the age at entry into the fishery at 2.5, we were slightly underharvesting the crop.

We believe other crayfish stocks could easily

lend themselves to management and that size limits alone would be sufficient to protect most stocks when combined with a limited entry program based on a lease arrangement for commercial harvest of water areas.

Acknowledgments

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Food and Feeding of Spiny Dogfish (*Squalus acanthias*) in British Columbia Waters

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JONES, B. C., AND G. H. GEEN. 1977. Food and feeding of spiny dogfish (*Squalus acanthias*) in British Columbia waters. J. Fish. Res. Board Can. 34: 2067–2078.

Stomach content data on 14,796 spiny dogfish (*Squalus acanthias*) from British Columbia waters are presented. The major dietary components based on occurrence were 55% teleosts, 35% crustaceans, and 5% molluscs. The principal food items were herring (22%) and euphausiids (14%). Prey was largely pelagic (80%), with fishes predominating in winter and invertebrates in summer. Fishes became more important in the diet with increasing dogfish size. Dogfish consumed twice as much food in summer as in winter. Annual consumption varied from 5 times biomass for small dogfish to 2.5 times for larger animals. Preliminary analyses suggest that dogfish consume over 5 times the current annual commercial catch of herring, but insignificant quantities of salmon.

Key words: dogfish, *Squalus*, feeding, food, biomass, predation, metabolism, seasonality, survivorship, digestion

JONES, B. C., AND G. H. GEEN. 1977. Food and feeding of spiny dogfish (*Squalus acanthias*) in British Columbia waters. J. Fish. Res. Board Can. 34: 2067–2078.

Les auteurs présentent les données sur les contenus stomacaux de 14 796 aiguillats communs (*Squalus acanthias*) des eaux de la Colombie-Britannique. Les composantes majeures du régime alimentaire, en terme d'occurrence, sont les téléostéeens (55%), les crustacés (35%) et les mollusques (5%). Les principaux item sont le hareng (22%) et les euphausides (14%). Les proies sont en grande partie pélagiques (80%), les poissons prédominant en hiver et les invertébrés en été. Les poissons deviennent de plus en plus importants dans le régime à mesure qu'augmente la taille de l'aiguillat. L'aiguillat commun consomme 2 fois plus de nourriture en été qu'en hiver. La consommation annuelle varie de 5 fois la biomasse chez les petits aiguillats à la moitié de ce chiffre chez les grands individus. D'après les résultats d'analyses préliminaires, l'aiguillat commun consommerait plus de 5 fois la prise commerciale annuelle courante de hareng, mais des quantités insignificantes de saumon.

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SPINY dogfish (*Squalus acanthias*) have long been viewed by British Columbia fishermen as major exploiters of commercially important marine organisms. Few data have previously been available to either refute or substantiate these claims. This study was designed to assess the feeding habits of dogfish and thereby attempt to clarify this issue.

Our approach was to determine the diet of dogfish from stomach content data based on occurrence frequency corrected for seasonal variation in consumption and food requirements in relation to size and sex. Several other workers (Razum 1952; Chatwin and Forrester 1953; Bonham 1954) have attempted to provide such dietary data for dogfish, but had only small samples of different sizes and sexes from each area and time of year. They also lacked data on the effect of availability to gear and gear selectivity on the observed diet and knowledge of rate of food consumption. We attempted to minimize these problems by bringing together as many stomach samples as possible, collected with all types of gear. Data on diet were converted by reference to consumption, biomass, and metabolism studies into mean annual consumption estimates for each food type per unit stock, and used to indicate possible impact of dogfish on species of importance to British Columbia commercial fisheries.

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Materials and Methods

Examination of stomachs from 14,796 dogfish caught in British Columbia waters with a variety of gear during this study and over the past 30 yr by staff of the Pacific Biological Station, Nanaimo, B.C., provided the dietary data (Table 1).

From counts of the occurrence of each recognizable diet item we determined its percentage occurrence

TABLE 1. Numbers of dogfish from British Columbia waters by size, sex, and season used in stomach content analysis. The three figures for each group–season category are (upper) the number of dogfish with empty stomachs, (middle) the total number of dogfish examined, and (lower) the number of occurrences of recognizable food. The stomach of each fish may contain more than one type of recognizable food plus unidentifiable animal remains and bottom materials. The percentage empty in each group is also indicated. Winter comprises January, February, and March; Spring, April, May, and June; Summer, July, August, and September; Fall, October, November, and December.

Size/Sex group	Length-group(cm)	Season				Total no.	Total empty (%)
		Winter	Spring	Summer	Fall		
		97	37	65	13	212	
Young	<46	233	218	182	39	672	31.5
		174	224	86	24	508	
		446	220	270	187	1123	
Immatures	46–78	753	912	1175	643	3483	32.2
		237	779	978	469	2463	
		279	162	96	237	774	
Subadult ♀	79–93	600	412	297	731	2040	37.9
		254	252	218	471	1195	
		926	342	721	347	2336	
Mature ♂	>78	1767	1096	2556	904	6323	36.9
		687	773	1874	521	3855	
		343	176	49	317	885	
Mature ♀	>93	918	416	146	798	2278	38.8
		500	246	100	458	1304	
		2091	937	1201	1101	5330	
Total		4271	3054	4356	3115	14796	36.0
		1852	2274	3256	1943	9325	

relative to the total number of all food types identified in the stomachs examined. Percentage occurrences were calculated separately for five apparently distinct groups of dogfish:

Young — males and females < 46 cm which form a relatively coherent pelagic group.

Immatures — males and females from 46 to 78 cm, separated mainly because of the distinctiveness of other groups.

Subadult females — from 79 to 93 cm, whose length fell between the 50% maturity sizes of males and females as noted for the following groups.

Mature males — above 78 cm, whose length exceeded that observed at the 50% maturity level established in Jones and Geen (1977b).

Mature females — above 93 cm, whose length exceeded that observed at the 50% maturity level established by Ketchen (1972).

These dogfish groups were defined on the basis of the length range that covered the majority of individuals in each group as suggested by Ford (1921) and our trawl surveys.

Seasonal variation in consumption was determined from voluntary feeding of a group of captive dogfish 60–111 cm long. These were taken by setline off Nanaimo and maintained in a 30 × 15 × 10-m deep netted enclosure in Departure Bay, B.C., from April 1974 to March 1975. They fed on herring suspended from a float line. Approximately 25% more individual herring than dogfish present were put out and replaced every 2 or 3 days, as preliminary study indicated this to be an adequate rate of supply. Numbers (ranging from 12 to 33) and weight of dogfish present in the enclosure were checked monthly. Consumption was recorded as daily weight of herring eaten per kilogram dogfish. We assumed that herring lost to other sources were balanced by consumption of other organisms in the enclosure by the dogfish.

Annual food intake requirements of dogfish were calculated from data on feeding of captive young, mean daily rates of consumption derived from studies in the marine enclosure, and growth rate estimates in Jones and Geen (1977a). Consumption rates for young were derived from five pups (taken from full term females caught by setline) held for up to 1.5 yr in a 0.6 × 1.2 × 0.9-m deep indoor fibreglass tank in which mean temperature was close to 10°C. They were allowed to feed voluntarily on pieces of sockeye salmon (*Oncorhynchus nerka*) fingerlings supplied daily. Dates of ingestions and mean weight consumed were recorded.

Stomach evacuation rates were determined from 75 dogfish 70–107 cm long caught by setline off Nanaimo during mid-June through August 1974. Up

to six dogfish at any one time were retained in 2-m diam by 1-m deep fiberglass tanks at about 10°C for 1 wk and were then force-fed whole herring or salmon (17–27 cm long). At specific times after feeding the dogfish were killed and stomach contents removed. Twenty-five dogfish had empty stomachs. The solid materials in all others were weighed after excess moisture had been removed; the difference in weight of solids before and after feeding was attributed to digestion. The calculated stomach clearance rates were subsequently applied to stomach capacity data for dogfish of similar size to estimate maximum annual consumption. Stomach volumes were determined by removing stomachs just after the animals were killed, tying off the pylorus, and filling the unsupported stomach with water to its junction with the esophagus (papilla region). No allowance was made for possible unnatural stretching of stomachs and no data were available for animals 30–65 cm.

Results and Discussion

Stomachs of 36.0% of dogfish examined were empty (Table 1). Variation in percentages of empty stomachs in the different groups will subsequently be used for an independent check of intake requirements. The percentage of those with food in their stomachs was minimal in winter and maximal in summer (Fig. 1). The 9466 dogfish with food in their stomachs contained a mean of 1.16 different types of food organisms (a total of 10,983 occurrences). Recognizable food types constituted 9325 occurrences, while the remainder consisted of 11.6% bottom materials (such as mud, stones, and plant material) and 3.5% unidentifiable animal remains.

In this work we attempted to develop a food budget for a unit stock of spiny dogfish as a basis for calculating impact on prey species. The stages in this process are summarized in model form for ease of understanding (Fig. 2). Among the characteristics used to derive the food budget was

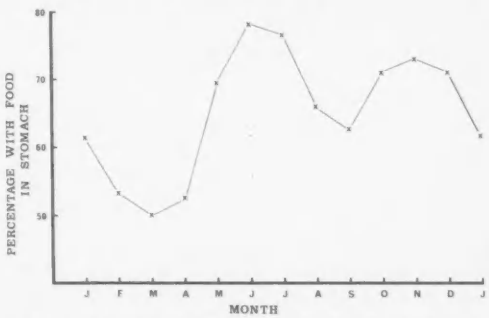

FIG. 1. Monthly variation in number of dogfish in British Columbia waters with food in stomach at time of capture. Curve smoothed by moving average of threes to clarify trend.

seasonal variation in consumption as derived from the marine enclosure data (Fig. 3). Food intake was greatest during summer and early autumn and least in late winter and early spring, a pattern observed for other sharks by Clark (1963). Dogfish consume twice as much in summer as in winter. The decrease in the October–November period corresponds to the breeding season when mature females move inshore, release their pups, and apparently cease feeding (Springer 1967). The difference between the mean maximum and minimum consumption levels during this period (Fig. 3) was 37%. This closely matches the proportional weight of mature females (33%) in the netted enclosure that might not be feeding during the breeding period. Mean daily consumption, as derived from the total integrated area under the curve (Fig. 3), was 1.3% of dogfish biomass. Relative consumption by season was derived from the integrated area under the curve for each season as a percentage of the total area (factor c in Table 2). The young and immature groups were assumed to exhibit the same seasonal changes in food consumption as adults, although they were largely unrepresented in the size-group kept in the marine enclosure.

The budget was also corrected for the proportional intake requirements of the different size-groups. For young kept in captivity, approximately 2.5 g/feeding were consumed just after birth, increasing to 13 g by 1 yr of age. A mean of 5.7 g was ingested every 2.6 days during the 1st yr of life. Mean initial weight of 55.2 g increased by 180% during this period, roughly 2.7 times the 66% weight increase normally encountered during the first year of life in the wild (Jones and Geen 1977a). However, there was no mean gain or loss in weight among dogfish in the marine enclosure. Thus, they were likely at a subsistence level of consumption. Applying a food conversion ratio of 12% [55.2 (180%/100)/5.7 (365/2.6)], derived from increase in weight of captive young relative to their total intake during the 1st yr of life, to the mean daily 1.3% body weight intake in the enclosure, the annual consumption by dogfish would be about 1.8 times $[(1 + 0.013(0.12))^{365}]$ their biomass. In the wild, weight increases 33.4%/yr for the young, decreasing to 3.1% for mature individuals (Jones and Geen 1977a). With a 12% food conversion ratio and assuming a 25% allowance for increase in activity for capturing prey, the annual intake requirement would be about 5 times [1.8 + 25% (1.8) + 33.4(1/0.12)/100] body weight for young decreasing to about 2.5 times for mature dogfish (factor b in Table 2). After compensation for 2.7 times greater growth rate than in

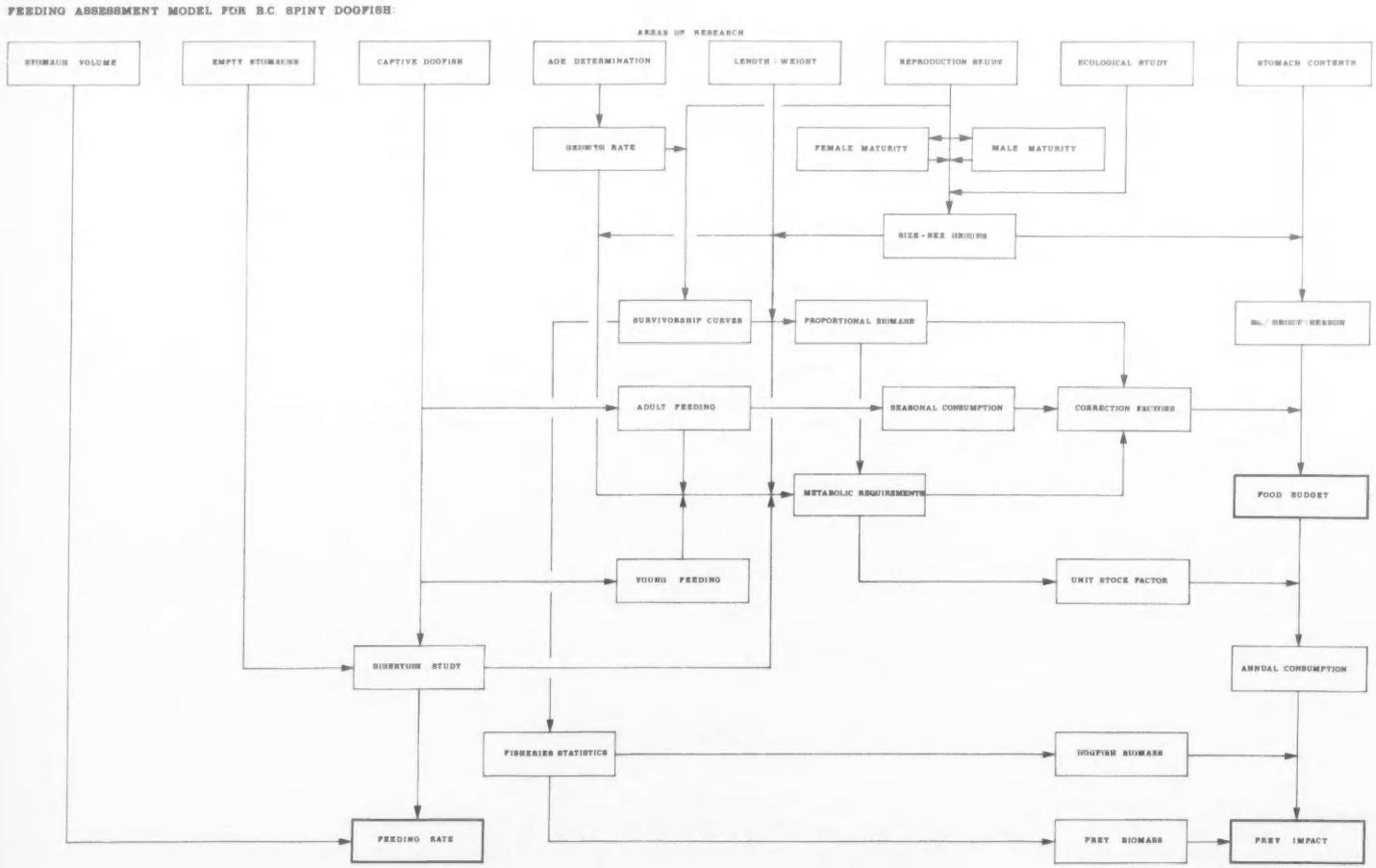

FIG. 2. Feeding assessment model for spiny dogfish in British Columbia waters to determine dietary proportions of prey (food budget), rate of feeding in the wild, and consequent impact on other species. Stages are more fully described in the text.

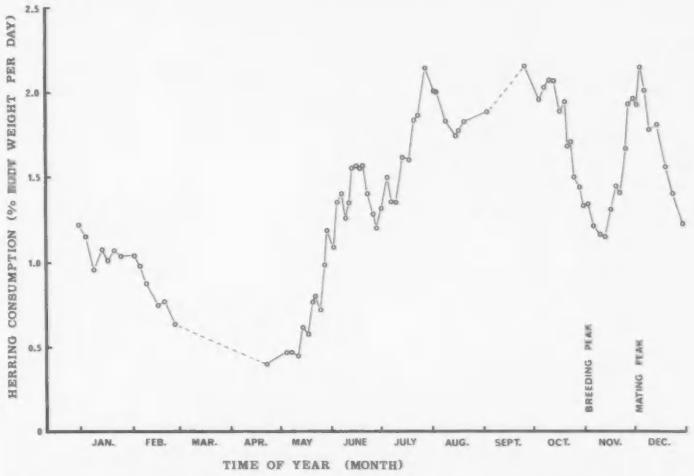

FIG. 3. Variation in herring consumption over 1 yr by dogfish held in a netted enclosure in Departure Bay, B.C. Each point is derived from a 5-point moving average. ○–––––○, periods for which no records are available.

TABLE 2. Correction factors developed for application in dogfish food budget calculations derived by combining terms for biomass, metabolic intake, and consumption according to season, size, and sex. Each figure in the group–season matrix is the percentage of the total annual stock intake consumed by dogfish in that category, and is derived by multiplying the corresponding three factors together (abc; same as footnotes) and reducing each product to a percentage of the sum of all categories.

Size/Sex group	Season				(a) Relative biomass (%)	(b) Intake requirement
	Winter	Spring	Summer	Fall		
Young	0.6	0.7	1.4	1.2	2.1	5.0
Immatures	2.0	2.2	4.3	3.8	10.4	3.2
Subadult ♀	4.3	4.8	9.3	8.3	26.9	2.7
Adult ♂	3.1	3.5	6.8	6.0	21.1	2.5
Adult ♀	6.0	6.8	13.2	11.7	39.5	2.6
(c) Relative consumption (%)	16	18	35	31	—	—

[a]Proportional biomass of groups in unit stock, as derived from mean numbers and weight per size (Fig. 6).

[b]Annual intake requirements (number of times own mean weight consumed) for groups as derived from food consumption and growth studies. Mean annual intake equals 2.719 times unit stock weight and is computed by the formula $\Sigma(ab/100)$.

[c]Seasonal consumption means derived from marine enclosure data (Fig. 3). Each value represents the integrated area under the curve for that season as a percentage of the total integrated area. The feeding patterns of all dogfish groups were assumed to be similar.

the wild, the annual intake for captive young equaled 5.4 times [5.7(365/2.6)/55.2(2.7)] their initial weight. This estimate was, as expected, slightly greater than the 5.0 found in Table 2 for the "young" dogfish group which would have a lower metabolic rate than 1st-yr individuals. Brett and Blackburn (1977) found that at a routine level of activity (slow cruising such as observed in the marine enclosure) a large dogfish utilized sufficient oxygen to require an annual food intake

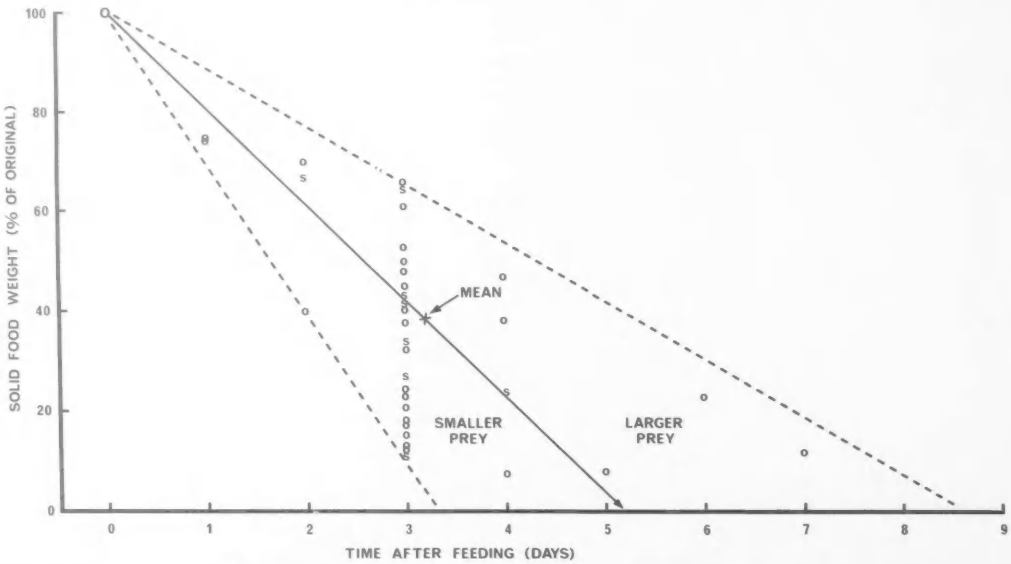

FIG. 4. Digestion rate of force-fed herring (○) in stomachs of dogfish from the Strait of Georgia, B.C. Mean herring weight was 120.3 g, requiring a calculated 5.16 days to be completely reduced to the fluid state. Data on digestion of sockeye salmon (s) are superimposed.

of 1.8 times body weight, a value similar to that determined from enclosure data.

To check intake requirements further, stomach clearance rates were examined and found to be independent of the size, sex, duration of captivity prior to testing, time of year, and food species and frozen storage time, although size of the food item had a significant influence (Fig. 4). For herring, a regression equation describing daily percentage digestion (D) on prey weight (W) was

$$D(\%) = 30.7 - 0.088W \text{ (g)}$$
$$(P < 0.05;\ r = 0.61;\ n = 27)$$

Mean herring weight was 120.3 g and a mean of 3.2 days was required to reduce this to 38.0% of its original weight. Plotting this point on Fig. 4 and extending a line from 100% and 0 days through the mean point to the abscissa indicated a stomach residence time of 5.16 days for complete breakdown of herring of this original weight to the fluid state. These data are then examined in conjunction with the variation in percentage of empty stomachs among dogfish groups (Table 1). When the variation in percentages of dogfish with food in their stomachs are derived from empty stomach data (Fig. 1) and compared with the variation in consumption by dogfish in the marine enclosure (Fig. 3), minima and maxima occur at approximately the same periods of late winter and summer, respectively, allowing a degree of time lag possibly relating to differential environmental influence between the marine enclosure and the Strait of Georgia. Since all contents in any one stomach were normally in the same state of digestion when examined in the field, and force-fed herring were always found to be retained in the stomach until fully broken down (this study; Hogben 1967), it is suggested that stomach residence time (the total food breakdown period) is inversely related to the variation in proportion of empty stomachs (representing the time between which food material has left the stomach and subsequent feeding). The mean percentage of empty stomachs (Table 1) increased from 31% for the young to 39% in the mature females. The lower percentages probably indicate a shorter stomach residence time inasmuch as smaller food organisms eaten by smaller dogfish likely require less time to digest than larger organisms. When the proportion of empty stomachs and rate of digestion as a function of size are known, it is possible to calculate the annual food intake. Mature males of mean length 85 cm had a mean weight of 2.15 kg and 36.9% empty stomachs (Table 1). It is assumed they would feed for 230 days [(1 − 0.369)365] of the year. With a digestion rate of 23.3 g [120.3/5.16] per day as derived from mean herring weight and total digestion time (Fig. 4), they would require an

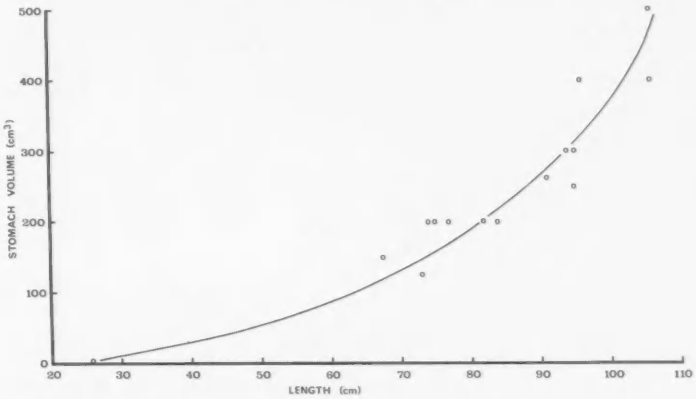

FIG. 5. Relationship of stomach volume to body length of dogfish from the Strait of Georgia, B.C. Curve fitted by eye.

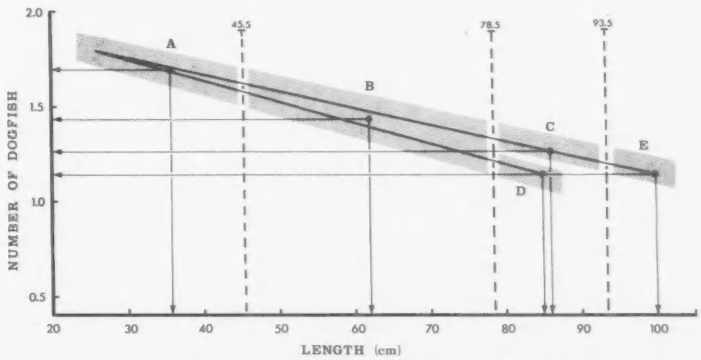

FIG. 6. Assumed relative survivorship curves for dogfish in British Columbia waters based on number surviving per mature female. Shaded areas delineate apparently distinct sex- and size-groups with the arrows noting their respective mean lengths and numbers. Curves end at modal length of matures, but in reality decrease rapidly beyond this length to zero at 103 and 130 cm for males and females, respectively. A = young, B = immatures, C = subadult females, D = mature males, and E = mature females. Based on data in Jones and Geen (1977b).

annual consumption of 2.5 times [(230 × 0.0233)/2.15] their weight which agrees with the estimate in Table 2. The stomach capacity of 85-cm dogfish is 223 cm³ (Fig. 5) which equals 234 g of herring (sp gr 1.044). Thus, mature male dogfish would take 10 days (234/23.3) to digest a full stomach of herring, take 16 days [10/(1 − 0.369)] between feedings, and feed only 23 times (365/16) per yr. This latter figure is minimal, as a dogfish may not completely fill its stomach on each feeding or it may eat smaller organisms at certain times which may require less time to break down. Holden (1966) estimated 40 feedings/yr. However, he did not specify the size range sampled. If they were generally smaller dogfish they would have higher metabolic rates and a proportionally greater food intake. For other species of sharks, Clark (1963) has estimated an annual intake of from 2 to 5 times body weight, being greater for the smaller sizes. Though Brett and Blackburn (1977) suggested an annual intake of 5 times body weight for adults, their growth rate assumptions were considerably in excess of those indicated by our data.

Another factor used in calculating the stock-food budget was the relative biomass of the different sex- and size-groups. Biomass estimates were calculated from mean weight and numbers derived

TABLE 3. Sample calculations of food budget and occurrence percentage for dogfish consumption of euphausiids in British Columbia waters. The top figure in each category (x) is the actual number of occurrences of stomachs containing euphausiids for that season, size, and sex category. The middle figure (y) is the percentage of euphausiid occurrences from among all occurrences of recognizable food within the category. The bottom figure (z) is the product of this percentage (y) and its corresponding correction factor from Table 2. Annual consumption is derived from correction of the segregated data for seasonal variation only (factor c in Table 2), to indicate variation in consumption among groups.

Size/Sex group	Season				Annual consumption (%)
	Winter	Spring	Summer	Fall	
Young	77 44.2 0.265	77 34.4 0.241	51 59.2 0.829	9 37.5 0.450	45.6
Immatures	20 8.4 0.168	268 34.5 0.759	247 25.3 1.088	55 11.7 0.445	20.0
Subadult ♀	2 0.8 0.034	78 30.9 1.483	45 20.5 1.907	23 4.9 0.407	14.3
Adult ♂	22 3.2 0.099	231 29.8 1.043	128 6.8 0.462	86 16.5 0.990	13.4
Adult ♀	1 0.2 0.012	23 9.4 0.639	10 10.0 1.320	9 2.0 0.234	5.8

Occurrence percentage = $\Sigma(x)(100)$/total occurrences
= 1462 (100)/9325 = 15.68%
Food budget = $\Sigma(z)$ = 12.87%

from survivorship curves (Fig. 6; based on data of Jones and Geen 1977b) and weight–length data in Jones and Geen (1977a). At modal length of mature individuals (100 cm) one breeding female produces 3.57 female pups annually (Jones and Geen 1977b). Since gestation takes 2 yr, only 1.79/yr are produced per mature female, but at modal length only 88% are mature (Ketchen 1972). Therefore, if the number mature at modal length is assumed to be one and a constant mortality rate is used, a survivorship curve for females originates with 1.79 pups at birth (26 cm) and decreases to 1.14 individuals (1/0.88) at 100 cm. Mature males have a modal length of 85 cm, but with only 87% mature at this length (Jones and Geen 1977b). Assuming a 50:50 sex ratio and that the numbers mature at the modal length are similar to those of mature females at modal length, a survivorship curve for males can also be constructed originating at 1.79 pups at birth and extending to 1.15 individuals (1/0.87) at 85 cm. When these curves (Fig. 6) are subdivided according to size, and mean numbers and lengths are derived for each dogfish group, relative biomass estimates can be calculated (factor a in Table 2). Though the curves imply a constant though greater mortality rate of males than females and a greater biomass of females than males, calculations indicated that if the survivorship curves were moderately convex or concave there would be little change in the relative biomass proportions.

The correction factors are combined on a relative basis in Table 2 to facilitate food-budget calculations. An example of their usage to determine the relative consumption of euphausiids by dogfish in British Columbia waters is shown in Table 3. The number of euphausiid occurrences for each category of size, sex, and season is converted to a percentage of all food-type occurrences for that category and multiplied by the corresponding combined correction factor in Table 2. The resultant percentages for all categories are summed and indicate that euphausiids constitute 12.87% of total stock diet. In comparison, the occurrence percentage of euphausiids in the diet calculated simply on frequency of occurrence is 15.68%, roughly 22% greater.

Our balanced food budget indicates that the major dietary components of British Columbia

TABLE 4. Mean annual percentages of food types consumed by different sizes and sexes of dogfish in British Columbia waters. The values derived in the food budget calculations are compared with the occurrence percentages. Only items with > 1% in at least one dogfish group are presented. Totals for major categories of food organisms are shown in parentheses. C = class, O = order, T = tribe, and F = family. For a comparison of ratios of budget to occurrence percentages, * = less than halved and ** = more than doubled from occurrence method.

Recognizable food type	Young (%)	Immature (%)	Subadult ♀(%)	Mature ♂(%)	Mature ♀(%)	Food budget (%)	Occurrence (%)
Plankton	(10.1)	(13.3)	(12.7)	(10.4)	(4.4)	(9.09)	(10.88)
Ctenophora							
Combjelly (*Pleurobrachia* sp.)	(1.7)	(1.7)	(2.3)	(2.8)	(2.2)	(2.26)	(2.34)
Coelenterata	(0.8)	(0.7)	(2.9)	(0.4)	(0.5)	(1.15)	(0.90)
Jellyfish (C. Scyphozoa)	0.8	0.7	2.7	0.4	0.5	1.10	0.87
Mollusca	(4.8)	(3.3)	(5.6)	(2.9)	(5.4)	(4.69)	(3.54)
Octopus (*Octopus* sp.)	0.1	1.6	4.3	2.0	3.0	2.87	2.14
Squid (*Loligo* sp.)	4.7	1.5	1.3	0.7	2.0	1.61	1.29
Annelida							
Worm (O. Polychaeta)	(5.2)	(2.0)	(0.6)	(1.0)	(1.1)	(1.22)	(1.63)
Crustacea	(60.4)	(33.9)	(28.2)	(27.3)	(22.4)	(27.78)	(31.02)
Amphipod (O. Amphipoda)	10.0	0.8	—	0.4	—	0.57*	1.47
Crab (T. Brachyura)	—	2.2	6.5	2.3	11.2	6.68	3.72
Euphausiid (O. Euphausiacea)	45.6	20.0	14.3	13.4	5.8	12.87	15.68
Shrimp (*Pandalus* sp.)	4.8	10.5	7.3	11.1	5.3	7.57	9.89
Echinodermata	(—)	(0.4)	(0.4)	(0.3)	(0.2)	(0.30)	(0.41)
Unident. invertebrate	(0.8)	(3.2)	(2.6)	(3.3)	(2.9)	(2.85)	(2.96)
Cyclostomata	(—)	(0.2)	(0.1)	(—)	(—)	(0.05)	(0.06)
Elasmobranchii	(—)	(0.2)	(2.0)	(0.4)	(3.6)	(1.99)**	(0.89)
Ratfish (*Hydrolagus* sp.)	—	0.1	1.4	0.3	3.2	1.65**	0.69
Teleostei	(16.2)	(41.1)	(42.6)	(51.2)	(57.3)	(48.62)	(45.37)
Herring (*Clupea* sp.)	0.5	10.6	10.3	23.0	15.6	14.42	16.33
Eulachon (*Thaleichthys* sp.)	13.4	10.2	2.6	3.0	1.6	3.65	3.23
Hake (*Merluccius* sp.)	—	1.7	5.0	0.9	7.3	4.48	2.68
Cod (F. Gadidae)	—	0.4	0.1	0.7	1.8	0.89	0.69
Flatfish (O. Heterosomata)	—	0.7	3.4	0.9	7.2	3.89**	1.64
Seaperch (F. Embiotocidae)	—	0.3	1.0	0.3	1.8	1.04**	0.38
Rockfish (F. Scorpaenidae)	—	0.4	1.0	0.7	1.4	0.98	0.60
Sandlance (*Ammodytes* sp.)	—	2.2	0.5	2.3	0.7	1.11	1.60
Unident. Teleostei	1.9	13.2	16.7	18.0	18.2	16.52	16.75

dogfish are 55% teleosts, 35% crustaceans, and 5% molluscs (Table 4). For the total stock, herring is the single most important food species (Table 5). Herring, shrimp, hake, and eulachon are the predominant food types consumed, constituting over 42% of the diet, while salmon is <0.3%. Fishes and invertebrates are consumed in approximately equal percentages. However, the dietary importance of each varies seasonally with dogfish size. Euphausiids are the major food of young and immature groups at all times of the year (Table 3, 4) and are important in the diet of larger dogfish during spring and summer. The diet of smaller dogfish is further supplemented by plankton, amphipods, and small fishes such as eulachon and herring. By the time dogfish reach 78 cm, herring rival euphausiids in importance and are the major food of larger individuals, particularly in fall and winter. Thus, as dogfish grow, the diet shifts from invertebrates towards fishes (Fig. 7). For larger animals, the greatest percentage of the diet of both sexes was fishes in winter and invertebrates in summer. Pattern irregularities for smaller sizes during the latter part of the year (Fig. 7) may be due to small sample sizes (Table 1). Dietary diversity was relatively lower among young dogfish (Table 4), possibly reflecting a limited ability to pursue and catch prey. On average over 80% of the food was pelagic. Food of smaller dogfish was obtained to a greater degree from the pelagic zone (86%) than that of mature males (78%) or mature females (70%) suggesting that mature dogfish are more demersal, an observation verified by trawl surveys (Jones 1974).

A ratio comparison of the food budget per-

TABLE 5. Individual food types in the food budget of dogfish in British Columbia waters ranked from highest to lowest. Annual consumption per unit stock (AC/US) is derived by multiplying these data by the proportional mean annual intake requirement of the stock as a whole (2.719 from Table 2).

Position	Food item	Diet amount (%)[a]	Cumulative total (%)	AC/US (%)[b]
1	Herring	21.84	21.84	59.4
2	Euphausiid	13.63	35.47	37.1
3	Plankton	9.65	45.12	26.2
4	Shrimp	8.03	53.15	21.8
5	Crab	7.09	60.24	19.3
6	Hake	6.79	67.03	18.5
7	Flatfish	5.89	72.92	16.0
8	Eulachon	5.53	78.45	15.0
9	Octopus	3.05	81.50	8.3
10	Combjelly	2.40	83.90	6.5
11	Squid	1.71	85.61	4.6
12	Sandlance	1.68	87.29	4.6
13	Ratfish	1.65	88.94	4.5
14	Seaperch	1.58	90.52	4.3
15	Rockfish	1.48	92.00	4.0
16	Cod	1.35	93.35	3.7
17	Polychaete	1.29	94.64	3.5
18	Jellyfish	1.17	95.81	3.2
19	Eelpout	0.67	96.48	1.8
20	Amphipod	0.60	97.08	1.6
21	Sculpin	0.35	97.43	1.0
22	Salmon	0.29	97.72	0.8
23	Dogfish[c]	0.28	98.00	0.8
24	Sablefish	0.27	98.27	0.7
25	Sea cucumber	0.25	98.52	0.7
26	Bivalve	0.22	98.74	0.6
27	Midshipman	0.15	98.89	0.4
28	Blenny	0.15	99.04	0.4
29	Prawn	0.14	99.18	0.4
30	Other	0.82	100.00	2.2

[a]Unidentified invertebrate and fish percentages prorated over respective groups.

[b]Percentage of its own weight that a unit stock annually consumes of any individual food type.

[c]Not likely representative of natural consumption, as it was observed that dissected dogfish thrown overboard soon appeared in stomachs of dogfish caught in subsequent trawls.

centages with the occurrence percentages from all data (Table 4) indicated that differences were as high as threefold. Though our approach is believed to provide more accurate estimates of dogfish diet and consumption rates than the uncorrected occurrence percentages, the following possible sources of bias are recognized:

(1) The frequency of occurrence method used assumes that occurrence percentages accurately reflect proportional biomass intake of individual food types. Since most stomachs contained only one food type (mean 1.16) and those containing food were often largely full, this bias is reduced though still of primary consequence when interpreting our results.

(2) The occurrence method also assumes that various food types are equally digestible. In this work we found that herring and salmon of the same size were digested at the same rate. Windell (1967) found bluegill (*Lepomis macrochirus*) of similar weight digested oligochaetes, arthropods, and fishes at the same rate. Even if dogfish food types are digested at the same rate, they may reach an unidentifiable condition at different times. Thus, higher proportions of soft-bodied types like ctenophores and coelenterates might become unidentifiable sooner than other organisms, which would underrate their actual dietary proportions. Similarly, species with hard parts which are difficult to digest, such as octopus and squid beaks, can be identified when digestion is far advanced. This source of bias is unaccounted for, though not likely of much significance, as few food types consumed by dogfish fall into this category.

(3) Much of our data further arise from opportunistic sampling in which many collectors took part. Thus, biases due to vessel purpose, gear selection, and differing individual ability to classify food species may exist. However, the multiplicity of samples collected over many years may have compensated for any error.

Bearing in mind the possible influence of these sources of error, the food budget percentages can be converted to percentages of its own weight that a unit stock annually consumes of any individual food type by multiplying by 2.719 (the mean annual intake requirement of the total stock in terms of number of times own weight consumed, Table 2; Table 5). From these data the annual consumption of commercial fisheries species can be calculated given an estimate of dogfish biomass. Johnson (1976) has estimated biomass in British Columbia waters at 0.387 million (range 0.3 to 0.5 million t). Dogfish consumption of commercial species (A) based on this estimate is indicated in Table 6 and compared with the 1974 commercial catch (B; Anon. 1975). Although the high A:B ratio for shrimp and prawn reflects low fishing effort for these species, this probably would not apply to major fisheries. Among the major commercial species, dogfish are estimated to have consumed 5.1 times as much herring but <0.1 times as much salmon. Though these figures certainly dispel the myth of the large consumption of salmon by dogfish, it cannot be denied that due to their opportunistic nature they will still eat salmon caught by line or net, and by so doing, waste fishing time and

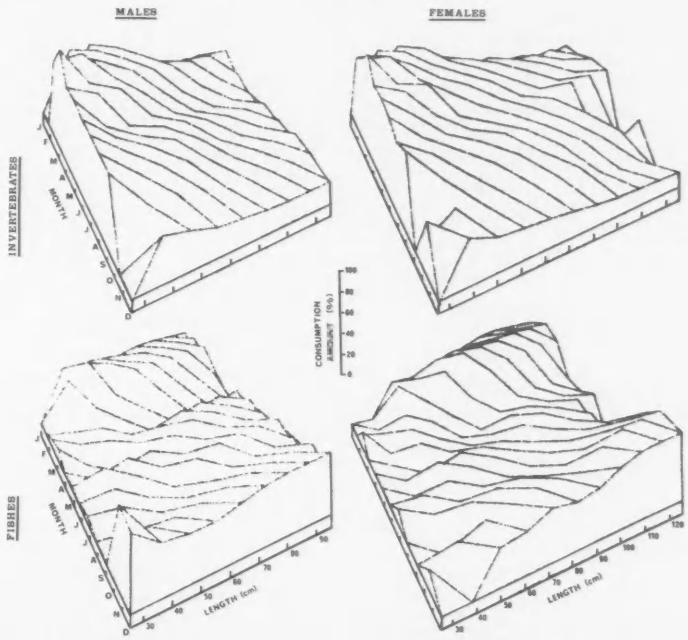

FIG. 7. Monthly consumption of invertebrates and fishes by dogfish in British Columbia waters in relation to size and sex. A 3-point moving average is applied to months and 10-cm length intervals to clarify data trends.

TABLE 6. Estimated annual consumption by dogfish of species of importance to commercial fishermen compared to the 1974 Canadian catch in British Columbia waters. This is calculated from a dogfish stock level of 0.387 million t (Johnson 1976) applied to the annual consumption per unit stock figures of Table 5.

Species harvested[a]	(A) Dogfish consumption (t, $\times 10^3$)	(B) 1974 Commercial catch (Anon. 1975) (t, $\times 10^3$)	A:B
Shrimp	84.4	1.17	72.1
Prawn	1.5	0.03	51.6
Crab[b]	37.4	1.14	32.8
Flatfish	61.9	7.78	8.0
Sablefish	2.7	0.34	8.0
Rockfish	15.5	2.68	5.8
Herring	229.9	44.66	5.1
Grey cod[c]	2.2	8.13	0.3
Salmon[d]	3.1	63.94	<0.1

[a]Ranked according to A:B ratio from highest to lowest.

[b]Commercial Dungeness crabs assumed 50% of total.

[c]Grey cod approximately 15% of cod (F. Gadidae) group.

[d]Includes sport fishery catch (approximately 1.5% by weight).

possibly destroy gear. Of more importance, however, is their effect on the herring fishery. Their calculated annual consumption of approximately 230,000 t of herring is in excess of record commercial herring catches off the British Columbia coast. With an adult herring stock level of 0.35 million t (Johnson 1976) and assuming a further 50% biomass for immatures, total British Columbia herring biomass approximates 0.53 million t. At the dogfish stock size indicated by Johnson (1976), our data would suggest an annual herring consumption level of 44% [0.23(100)/0.53] of total stock. The range of sampling error would likely only reduce this estimate by up to 50%, still a significant annual consumption of herring. Needless to say, reduction in dogfish numbers does not automatically imply that large increases of particular species such as herring would be commercially available, as other predators, which are themselves preyed upon by dogfish, may also increase in numbers and check the expansion of such species.

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Growth of Rock Bass, *Ambloplites rupestris*, in Relation to the Morphoedaphic Index as an Indicator of an Environmental Stress[1]

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RYAN, P. M., AND H. H. HARVEY. 1977. Growth of rock bass, *Ambloplites rupestris*, in relation to the morphoedaphic index as an indicator of an environmental stress. J. Fish. Res. Board Can. 34: 2079–2088.

Atmospheric contamination by sulfur dioxide in the Sudbury region and the associated acidification has reduced population densities of fishes in the La Cloche Mountain lakes, 33–90 km away. Surviving rock bass (*Ambloplites rupestris*) responded to decreased abundance with increased growth rate. Variation in abundance among surviving populations could be monitored by means of individual growth rates and mean lake depth. Our findings suggest that growth rate of fishes, in conjunction with the morphoedaphic index, may serve as an indicator of environmental stress.

Key words: environmental indicator, environmental stress, lake acidification, fish growth rate, morphoedaphic index, population density, rock bass

RYAN, P. M., AND H. H. HARVEY. 1977. Growth of the rock bass, *Ambloplites rupestris*, in relation to the morphoedaphic index as an indicator of environmental stress. J. Fish. Res. Board Can. 34: 2079–2088.

La contamination atmosphérique par le bioxyde de soufre dans la région de Sudbury et l'acidification qui lui est associée ont diminué les densités de population de poissons dans les lacs de la montagne La Cloche, à une distance de 33–90 km. La réponse du crapet de roche (*Ambloplites rupestris*) à une diminution d'abondance a été une augmentation du rythme de croissance. Les rythmes de croissance individuelle et la profondeur moyenne des lacs ont permis de suivre la variation dans l'abondance des populations qui survécurent. Nos observations suggèrent que le rythme de croissance des poissons, couplé à l'indice morphoédaphique, peut servir d'indicateur de stress écologique.

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THE value of the morphoedaphic index as a predictor of actual fish yield was established by Ryder (1965) and reviewed by Ryder et al. (1974), who noted it was influenced by anomalous environmental conditions in the lakes considered. Thus, in lakes where actual yield deviates markedly from predicted yield, anomalous environmental conditions can be suspected. Generally, among lakes possessing similar carrying capacities or similar levels of potential productivity, those with fewer fish per unit volume or area can be expected to possess faster-growing individuals. Thus, it can be expected that variation in abundance or population density in fish populations can be identified by means of the readily quantified growth rate of individuals plus the morphoedaphic index.

[1]Based in part on a thesis submitted by P. M. Ryan in conformity with the requirements for the degree of Master of Science in the University of Toronto.

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Entire fish populations have been lost or reduced in the La Cloche Mountain lakes of Ontario, through a process of atmospheric contamination and lake acidification (Beamish and Harvey 1972; Harvey 1975). This report describes the growth responses of La Cloche rock bass (*Ambloplites rupestris*) to lake acidification and how growth rate, taken in conjunction with the morphometric component of the morphoedaphic index, served to differentiate between lakes undergoing varying degrees of environmental stress.

Materials and Methods

From 1966 to 1974 data were collected on the age and growth of rock bass, plus physical, chemical, and biological parameters in 25 La Cloche lakes.

LAKE DESCRIPTION

Four descriptive lake statistics are considered: mean depth, total dissolved solids, the morphoedaphic index itself, and pH as the measure of environmental stress (Table 1).

TABLE 1. Descriptive data for the 25 La Cloche lakes with populations of rock bass. Chemical measurements performed on surface water samples taken from May to October 1970–74.

Lake	Lat.	Long.	pH	Total dissolved solids (mg/ℓ)	Mean depth (m)	Morphoedaphic index
Charlton	460815	814245	6.48[a]	30[a]	2.4	12.5
Little La Cloche	460925	820458	6.36[a]	29	1.2	24.2
Evangeline	460815	815200	6.31[a]	29	5.2	5.6
La Cloche	460830	820430	6.17[a]	29[a]	16.3	1.8
Alexander	460657	814930	6.16[a]	21[a]	9.0	2.3
Bodina	460630	813000	6.16[a]	22[a]	2.1	10.5
Howry	460915	812800	6.14[a]	20[a]	14.2	1.4
Low	460000	813400	6.10	39[a]	16.3	2.4
Florence	460702	814800	6.05[a]	20[a]	11.5	1.7
Frood	460737	814020	6.04[a]	29[a]	5.4	5.4
Hele	460700	813400	5.97[a]	22[a]	21.6	1.0
Fish	461000	812300	5.76[a]	18[a]	3.6	5.0
Kakakise	460345	811915	5.73[a]	21	12.2	1.7
Gem	460930	812615	5.66[a]	20[a]	9.8	2.0
Ishmael	460700	813500	5.51[a]	20[a]	12.5	1.6
Little Sheguindah	460140	812328	5.18[a]	20[a]	1.7	11.8
Three Narrows	460630	812630	5.14[a]	20[a]	13.1	1.5
Carlyle	460330	811700	4.96[a]	20	5.8	3.5
Bell	460800	811145	4.88[a]	23[a]	8.0	2.9
Johnnie	460600	811400	4.83[a]	23[a]	7.9	2.9
Owl	460810	820775	4.82[a]	17	0.9	18.9
Killarney	460400	812130	4.61[a]	22[a]	9.7	2.3
George	460145	812400	4.60[a]	22[a]	18.6	1.2
Great Mountain	460920	812245	4.48[a]	20[a]	9.8	2.0
Muriel	460307	812600	4.36[a]	24	7.0	3.4

[a]Average of two or more determinations made on samples drawn from different locations and/or at different times. Average pH calculated as the negative logarithm of the average hydrogen ion concentration.

In the field, pH was measured on surface water samples as soon as possible after they were drawn, generally within a few hours, using a portable pH meter in conjunction with a Sargent–Welch combination electrode or a Corning low-ionic pH electrode plus reference electrode. Measurements for Ishmael and Hele lakes were provided by N. Conroy (Ontario Ministry of Environment). Where two or more pH measurements were made at different times or on different locations on a lake, they were converted to hydrogen ion concentrations, averaged, and converted back to pH.

FISH GROWTH

Each of the 25 lakes was fished with 50-m multifilament gill nets containing six panels of 38-, 50-, 60-, 66-, 76-, and 90-mm stretched mesh. In most lakes, one or more of commercial-type gill nets ranging in stretched-mesh from 19 to 90 mm, fine-mesh trap nets (Beamish 1972), hoop nets, plastic MKII traps (Casselman and Harvey 1973), and a chemical fish collector were used to catch rock bass. Total lengths and scale samples were obtained routinely from the catch or, rarely, a representative subsample to provide information on age and growth.

Scales were examined and measured on an Eberbach microprojector at a magnification of 39 diameters. An age was assigned to each fish equal to the number of completed scale annuli using the criteria for annulus identification described by Tesch (1971). Fish in their 1st yr of life were considered to be members of age-group 0 or age 0, fish in their 2nd yr were members of age-group 1 or age 1, and so on.

A scale from each fish, regular in shape and appearance, was measured from the focus to the last completed annulus and to the scale edge along the anteriormost radius. The following linear relationship between fish length in millimetres (Y) and magnified anteriormost scale radius in millimetres (X) was determined using least-squares regression: $\log_n Y = 1.65 + 0.72 \log_n X$, where $N = 1219$ and $r = 0.969$. Using this line, Fraser's (1916) proportionality formula, and the scale measurements, the length of each fish at the end of its last full year of life was calculated. This procedure compensated for apparent differences in growth resulting from the different times of capture of the fish. Those statistics were used to compute the mean length of the fish in each age-group by lake and year of capture. The mean number of annuli (age) and mean length at capture were calculated for each population sampled.

To make comparisons easier, the growth statistic, mean age at 110 mm, was derived to quantify the

growth rate in each population. Mean calculated lengths at age were plotted against age, adjacent means joined by straight lines, and mean lengths corresponding to the youngest ages connected to the origin of the coordinated axes. The youngest age corresponding to the selected length was then read, to the nearest tenth of a year, from the abscissa, yielding a continuous variable inversely proportionate to growth rate. The one population not described by this statistic was represented by one fish.

Selection of the length used to compute the growth statistic was based on several interdependent factors. Rock bass with calculated lengths of 110 mm were comparatively young, and therefore ages were more likely to be assigned correctly. Because the fish were young, these individuals related more closely to the conditions in the lake measured at the time of capture. Fish of this length were the smallest individuals captured readily by gill nets and since gill nets were used most frequently this gear accounted for most of the rock bass caught. This length generally corresponded to an even greater length at capture, thus partially compensating for gillnet selectivity and a possible underestimation of the mean age at a given length resulting from such selectivity. Sexual differentiation in growth at this length tended to be relatively small.

Analysis of Data

Variations in growth, abundance, and reproductive success were examined with respect to the lake descriptive data by comparing mean lengths at each age, mean ages at 110 mm, mean length at capture, mean ages, and the frequencies of occurrence of young-of-the-year fish (Table 2). Analysis was by least-squares regressions yielding best-fitting linear, exponential, power, and hyperbolic functions, and by least-squares, stepwise, linear, multiple regressions (Nie et al. 1970).

Results and Discussion

Reduced Growth Rate

The age–length relationship of rock bass taken from George Lake in 1966 was compared with that of rock bass taken from the lake in 1973 and 1974 (Fig. 1). It was concluded that a reduction in the rate of growth of rock bass had occurred over the period and was being followed by an increase in the rate of growth of the most recent year-classes. In George Lake these changes in rock bass growth were concurrent with a more than tenfold increase in hydrogen ion concentration, from pH 5.78 in 1966 (from the predictive equation of Beamish et al. 1975) to 4.60 in 1970–74.

A reduction in the rate of growth of fishes is a symptom of environmental stress in the early stages of acidification of lakes. There are many examples of the poor growth of fishes in acid waters, two of which are especially pertinent to the situation in George Lake. Eddy and Carlander (1942) found a positive correlation between the growth rate of rock bass and lake pH within a large group of Minnesota lakes with acid levels not lethal to fishes. Beamish et al. (1975) reported a marked decrease in the growth rate of white suckers (*Catostomus commersoni*) in George Lake from 1967 to 1972.

This phenomenon, as exemplified by the George Lake rock bass of age-group 4 and over, cannot be attributed to reductions in food supplies. Many species of invertebrates, the principal food source of rock bass (Keast and Webb 1966), are abundantly represented in acid waters generally, (EIFAC 1968; Bell 1971) and La Cloche lakes in particular (Beamish 1970; Sprules 1975). Reduced growth may be due to such factors as the impairment of respiratory processes, possibly via the "coagulation film anoxia" of Douderoff and Katz (1950), or the disruption of normal circulatory processes by a lowered blood pH, the "acidaemia" of Lloyd and Jordan (1964). Given the same available food supply per individual, but a higher energy loss due to disruption of these processes, it can be expected that the growth rate of fishes would decrease initially during lake acidification.

Increased Growth Rate

The growth of individuals tended to be faster in lakes of greater acidity (Table 3). The best-fitting linear descriptor of the relationship of mean length at age to lake pH was negative in slope in all cases except age-groups 9 and 12. These negative slopes were significant ($P \leq 0.05$) only in age-groups 2, 4, and 5. However, mean lengths at age 1 were distributed over only a small range of the independent variate. Also, the relationship of mean length at age 3 to lake pH was approaching significance at the 5% level, but the inclusion of the atypically fast-growing Bodina Lake rock bass weakened the correlation. As the number of data points increased in age-groups 4 and 5, and the data were distributed over a broader range of the independent variate, the slopes became significant; in age-groups 6 and older, none of the slopes were significantly different from zero.

Solution to the linear regressions of mean length at each age on lake pH through age-groups 1–8 provided a model predictive of growth at three levels of stress encountered by the La Cloche populations (Fig. 2). Differences in growth rate were most obvious in the younger age-groups. The majority of the regressions of

TABLE 2. Age and growth data of rock bass (sexes combined) from 26 populations in 25 La Cloche lakes. All fish, except for those from George Lake were captured from May to October, 1972 to 1974. The lake pH corresponding to each population is presented. Shown below the mean are, where applicable, the 95% confidence interval about the mean and the sample size in parentheses.

Lake	Lake pH	Mean total length (mm) at end of life in age-group												Mean age at 110 mm (yr)	Mean total length at capture (mm)	Mean no. of annuli
		1	2	3	4	5	6	7	8	9	10	11	12			
Charlton	6.48	37.6 (1)	62.2 ±4.6 (10)	86.5 ±23.0 (4)	109.8 ±6.3 (15)	135.8 ±5.3 (13)	153.2 ±8.2 (18)	164.1 ±18.0 (2)		228.0 (1)				4.0	125.7 ±10.5 (70)	4.1 ±0.5 (70)
Little La Cloche	6.36	35.6 ±6.8 (5)	59.3 ±24.1 (2)		117.1 ±7.5 (6)	134.8 ±6.3 (8)	166.5 ±9.9 (7)	183.7 ±13.6 (7)		2.115 ±34.1 (3)				3.8	118.4 ±18.8 (51)	3.7 ±0.8 (51)
Evangeline	6.31	42.5 ±7.1 (4)	62.5 (1)	79.6 ±17.0 (3)		129.4 ±23.3 (3)	135.2 ±22.5 (3)	160.0 ±239.5 (2)	153.8 (1)	210.3 (1)	209.4 ±77.5 (2)			4.2	84.8 ±21.8 (36)	2.8 ±1.1 (36)
La Cloche	6.17					109.1 ± (1)	136.7 ±49.8 (3)		200.2 ±24.9 (3)	223.7 ±8.8 (9)				5.0	203.6 ±20.5 (16)	8.0 ±0.8 (16)
Alexander	6.16	44.8 ±3.6 (9)	71.1 ±1.5 (12)	90.9 ±4.6 (8)	104.4 ±2.7 (10)	115.6 ±8.2 (9)	118.2 ±4.0 (10)	122.5 ±6.4 (5)	149.5 ±139.1 (2)	157.7 ±219.8 (2)	154.0 (1)	257.7 (1)	259.2 (1)	4.5	107.7 ±9.4 (70)	4.3 ±0.6 (70)
Bodina	6.16			121.1 ±5.4 (22)	146.7 ±7.6 (5)	155.6 ±59.1 (2)	187.5 ±4.3 (16)	207.3 ±9.0 (7)						2.7	171.3 ±7.9 (52)	4.6 ±0.4 (52)
Howry	6.14			89.1 (1)	97.4 ±7.1 (8)	118.1 (1)	128.7 ±12.4 (8)	141.1 (1)		178.6 (1)				4.6	120.1 ±14.1 (21)	5.0 ±0.8 (21)
Low	6.10	32.7 ±2.1 (12)	53.5 ±5.4 (5)		95.3 ±3.2 (14)	110.1 ±5.3 (12)	130.1 ±6.7 (11)	149.4 ±10.8 (10)	168.1 (1)	169.7 (1)				5.0	109.0 ±10.2 (66)	4.4 ±0.5 (66)
Florence	6.05	36.9 ±3.3 (6)	64.7 ±3.4 (5)	84.9 ±3.5 (12)	95.8 ± (15)	107.9 ±4.2 (10)	116.7 ±5.6 (12)	129.7 ±3.3 (13)	145.5 ±6.2 (4)	165.8 (1)				5.21	106.6 ±6.2 (78)	4.7 ±0.5 (78)
Frood	6.04			66.7 (1)	88.5 ±14.0 (5)	120.3 ±7.4 (6)	152.0 ±67.1 (3)	156.4 ±26.3 (5)	188.1 ±27.8 (4)	226.8 ±43.5 (3)	170.2 (1)			4.7	135.8 ±22.0 (33)	5.3 ±1.0 (33)
Hele	5.97	34.8 ±34.3 (2)			94.6 ±3.1 (20)	106.3 ±3.9 (16)	115.4 ±4.5 (9)	132.7 ±7.1 (9)	143.4 ±15.0 (4)	154.8 ±29.6 (3)	162.5 ±7.8 (3)	178.4 ±95.9 (2)		5.4	117.6 ±7.2 (70)	5.6 ±0.5 (70)
George 1966	5.78 a			94.9 ±7.8 (7)	119.0 ±9.1 (8)	146.3 ±5.7 (21)	161.9 ±7.6 (9)	184.5 ±24.8 (2)	191.4 ±12.8 (3)	195.8 (1)	216.4 ±39.4 (2)			3.6	153.2 ±8.2 (53)	5.3 ±0.5 (53)
Fish	5.76		76.2 (1)	96.8 (1)	124.1 ±12.3 (4)	128.0 ±4.6 (7)	133.1 ±5.4 (3)	157.2 ±6.5 (6)	180.2 (1)		244.6 (1)			3.5	150.0 ±11.8 (24)	5.6 ±0.7 (24)

TABLE 2 (*Concluded*)

Kakakise	5.73		81.9 (1)	94.0 ±5.5 (9)	101.8 ±3.3 (11)	115.7 ±5.0 (18)	124.7 ±4.8 (24)	130.5 ±11.4 (9)	135.1 ±15.2 (3)	141.1 (1)				4.6	125.0 ±3.1 (76)	5.3 ±0.3 (76)
Gem	5.66	39.5 ±2.2 (11)	69.1 ±4.2 (22)	88.0 ±6.5 (5)	109.6 ±4.6 (12)	129.4 ±7.7 (9)	144.1 ±28.1 (4)	154.1 ±6.8 (8)	167.6 ±9.3 (7)	193.3 ±0.6 (3)	190.0 ±17.8 (2)			4.0	111.8 ±10.0 (90)	3.8 ±0.6 (90)
Ishmael	5.51		53.4 ±9.5 (4)	79.3 ±4.2 (9)	92.5 ±5.8 (10)	104.4 ±6.5 (12)	130.6 ±7.9 (15)	171.8 ±20.3 (3)						5.2	115.3 ±8.3 (53)	4.6 ±0.4 (53)
Little Sheguindah	5.18		84.7 ±52.1 (2)		127.1 ±43.8 (2)									3.0	122.8 ±32.9 (4)	3.0 ±1.8 (4)
Three Narrows	5.14	43.9 ±14.0 (2)		96.6 ±28.8 (3)	108.3 ±11.6 (6)	126.0 ±11.8 (8)	141.5 ±5.3 (12)	161.5 ±6.2 (9)	173.2 ±9.9 (7)	194.2 ±16.4 (4)				4.1	137.4 ±12.6 (57)	5.3 ±0.7 (57)
Carlyle	4.96						158.0 ±117.5 (2)	176.6 ±9.2 (7)	189.6 (1)	182.2 (1)				4.2	183.7 ±9.7 (11)	7.1 ±0.6 (11)
Bell	4.88					155.5 ±5.9 (25)	169.4 ±10.0 (17)	180.6 ±9.3 (9)	181.6 ±34.5 (4)					3.5	175.5 ±4.9 (55)	5.9 ±0.3 (55)
Johnnie	4.83					158.2 ±16.2 (4)	174.1 ±48.3 (2)	194.8 ±11.8 (6)	200.4 ±14.5 (9)	214.1 ±33.6 (3)				3.5	201.4 ±9.5 (24)	7.2 ±0.5 (24)
Owl	4.82				174.6 (1)	201.1 ±12.8 (15)	212.3 ±52.4 (3)	228.5 ±42.0 (4)	258.8 ±9.3 (3)	259.4 ±8.2 (3)	269.1 ±36.1 (2)	284.1 (1)		2.5	228.5 ±16.0 (33)	6.3 ±0.8 (33)
Killarney	4.61		83.8 ±54.6 (2)		122.5 (1)	165.7 ±16.6 (6)	162.7 (1)	172.6 ±4.6 (2)		166.1 (1)				3.4	163.3 ±17.3 (13)	5.2 ±1.2 (13)
George 1973-74	4.60			103.7 ±24.2 (3)	110.3 ±2.7 (23)	124.5 ±5.3 (22)	130.0 ±4.7 (31)	144.8 ±8.0 (11)	160.7 ±17.2 (9)	171.3 ±23.6 (5)	173.2 ±9.7 (5)	180.3 (1)	168.7 (1)	3.8	137.7 ±4.2 (111)	6.0 ±0.3 (111)
Great Mountain	4.48									202.7 (1)					205.0 (1)	9.0 (1)
Muriel	4.36		94.6 ±18.4 (2)	112.6 (1)	144.2 ±9.6 (8)	148.2 ±5.4 (18)	156.5 ±5.7 (8)	162.8 ±12.0 (5)	171.5 ±6.7 (4)	179.0 ±23.5 (4)	201.1 (1)			2.8	159.4 ±5.2 (51)	5.7 ±0.5 (51)
All populations Unweighted mean		38.4 ±1.6 (52)	67.8 ±2.6 (69)	96.9 ±3.7 (89)	107.1 ±2.4 (184)	134.3 ±3.4 (246)	142.8 ±3.2 (231)	159.7 ±4.6 (142)	175.8 ±6.9 (70)	198.0 ±8.6 (52)	194.7 ±16.3 (20)	215.8 ±63.9 (5)	213.9 ±574.9 (2)	4.1	134.4 ±2.7 (1219)	5.0 ±0.1 (1219)
All populations Mean of means		38.7 (9)	70.5 (13)	92.3 (15)	114.2 (20)	132.4 (23)	147.5 (24)	163.8 (23)	175.5 (18)	191.7 (21)	199.1 (10)	225.1 (4)	214.0 (2)	4.0 (25)	145.0 (26)	5.3 (26)

[a]Value obtained from Beamish et al. (1975).

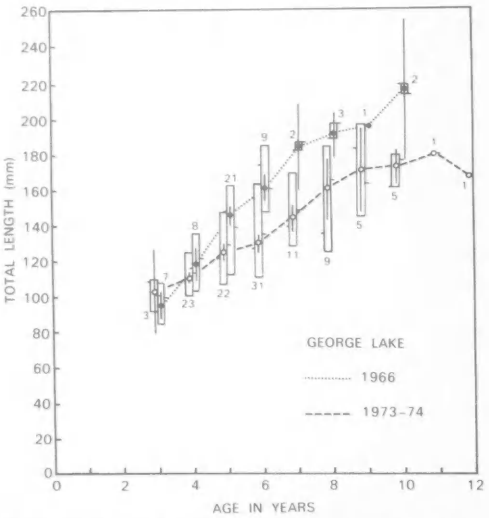

FIG. 1. Size at age of rock bass sampled from George Lake in 1966 and 1973–74. Circles are means, vertical lines are 95% confidence limits, rectangles are ranges, mean length of males and females are shown by horizontal lines to the right and left of the ranges, respectively, numerals indicate number of fish examined.

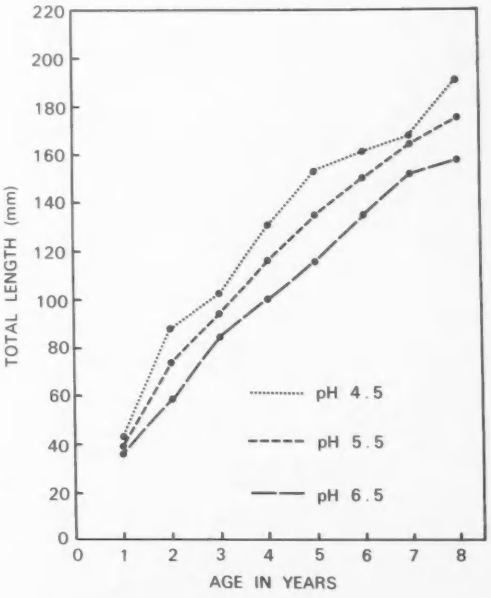

FIG. 2. Predicted age–length relationships of rock bass at three levels of pH in the La Cloche lakes. Lengths at each age were predicted by linear regression of mean length at age on lake pH.

mean length of rock bass at age against lake pH were described best by curvilinear functions, but because the increases in the correlation coefficients were small, simpler linear functions were retained here.

The use of the growth statistic, mean age at 110 mm, helped clarify the relationship between the growth rate of individuals in the younger age-groups and lake pH (Fig. 3). Since estimates of this value were obtained from all except one population, a better description of the relationship was obtained than was possible employing the less numerically represented, mean lengths at age. The relationship between mean age at 110 mm and lake pH could be described by a significant ($P = 0.01$) linear function of positive slope with 27% of the variance in the growth statistic accounted for by lake pH. On the average, rock bass that grew in a lake of pH 4.4 took 3.3 yr to reach a length of 110 mm whereas those that grew in a lake of pH 6.4 took 4.6 yr to reach the same length.

It was hypothesized that the mechanism causing increased growth rates at higher acid levels was reductions in population densities due to reproductive failures. Populations of rock bass in the more acid lakes tended to be comprised of larger, older fish or conversely, were characterized by fewer small, young fish.

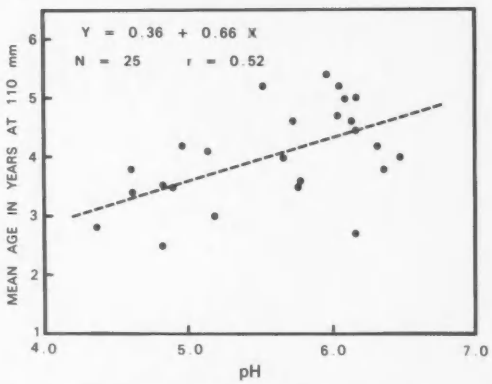

FIG. 3. Relation between lake pH and mean age of rock bass at a length of 110 mm.

Data were available from 20 lakes in which applications of rotenone had been made specifically for the capture of small fish. A comparison of the percentage of these lakes that yielded rock bass of age-group 0 indicated a lower rate of reproductive success in the more acid lakes (Fig. 4). The most acid lake yielding young-of-the-year rock bass was characterized by a pH of 4.82. A single fish of age-group 0 was captured in Owl

TABLE 3. Results of least-squares curve-fitting procedures to determine the relationship between lake pH and rock bass growth. Data for the sexes are combined. *P ≤ 0.05; **P ≤ 0.01.

Dependent variate[a]	Number of data points	Linear regression of the dependent variate on lake pH			Curve of best fit[b]
		Y intercept	Slope	Correlation coefficient	
Mean length					
age 1	9	60.24	−3.58	−0.345	6
age 2	13	155.61	−14.89	−0.760**	3
age 3	15	148.92	—9.89	−0.449	6
age 4	20	204.97	−16.13	−0.475*	6
age 5	23	241.53	−19.36	−0.537**	4
age 6	24	223.28	−13.52	−0.370	4
age 7	23	234.72	−12.70	−0.323	2
age 8	18	272.03	−17.50	−0.386	1
age 9	21	186.38	0.96	0.023	1
age 10	10	283.09	−15.15	−0.284	1
age 11	4	225.76	−0.12	−0.002	4
age 12	2	−98.16	58.01	Not Applicable	1
Mean age in years at 110 mm	25	0.36	0.66	0.518**	4
Mean length at capture of aged fish	26	320.35	−31.60	−0.571**	2
Mean number of annuli of aged fish	26	11.41	− 1.10	−0.515**	6

[a]Length are total lengths in millimetres.

[b]1. $Y = A + BX$ A linear function

2. $Y = Ae^{BX}$ An exponential function

3. $Y = AX^{B}$ A power function.

4. $Y = A + \frac{B}{X}$ A hyperbolic function.

5. $Y = \frac{1}{A + BX}$ A hyperbolic function.

6. $Y = \frac{X}{AX + B}$ A hyperbolic function.

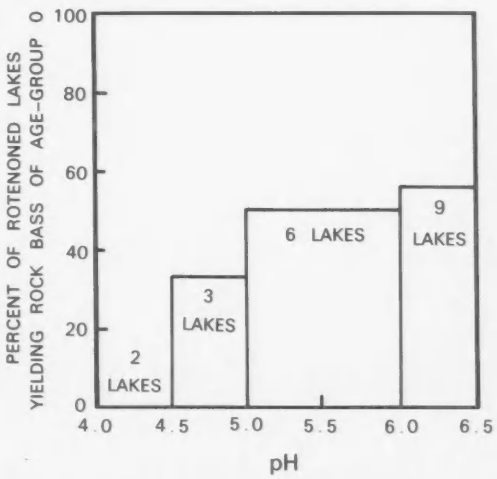

FIG. 4. Frequency distribution of age-group 0 rock bass in La Cloche lakes over pH 4.0–6.5.

Lake in September of 1973 when the lake pH was 4.90. In lakes characterized by a pH of 5.01–5.50, only one rock bass population was subjected to applications of rotenone. This was Three Narrows Lake which yielded six age-group 0 rock bass, possibly as a consequence of a localized increase in pH. Of the 20 populations subjected to rotenone applications, nine yielded fish of age-group 0. Five of these, or 56%, were in lakes of pH higher than 6.0. Of the 57 age-group 0 fish captured, only 13, or 23%, came from lakes of pH less than 6.0. These results were consistent with the negative correlations of mean length at capture of aged fish, and mean age of fish on lake pH.

In the later stages of lake acidification there is an increase in the growth rate of nonpiscivorous fishes due to reductions in population densities and increases in the available food supply per individual. The mean length of age-group 3 rock bass captured in acidifying George Lake in 1973 and 1974 was greater than the mean length of the

TABLE 4. Correlation matrix of five variates from 24 La Cloche lakes. All variates, except pH, were logarithmically ($\log_{10}$) transformed prior to analysis by least-squares, linear regression. $^{*}P \leq 0.05$; $^{**}P \leq 0.01$.

	Mean age of rock bass at 110 mm (yr)	Mean depth (m)	Total dissolved solids (mg/ℓ)	Morpho-edaphic index	pH
Mean age in years of rock bass at 110 mm	1.000				
Mean depth (m)	0.683**	1.000			
Total dissolved solids (mg/ℓ)	0.300	0.078	1.000		
Morphoedaphic index	−0.613**	−0.975**	0.144	1.000	
pH	0.525**	−0.056	0.432*	0.147	1.000

same age-group captured in the lake in 1966. Rock bass in the La Cloche region as a whole tended to grow faster in lakes of greater acidity.

Increased growth rates of nonpiscivorous individuals in acid waters have been documented previously. Frost (1940) reported the growth of rainbow trout (*Salmo gairdneri*) in a lake of pH 4.8 was much better, but only during the first 2 yr of life, than was the growth in three lakes of pH 6.8–8.4. Bluegill sunfish (*Lepomis macrochirus*) in an isolated stream pool varying near pH 4.5 showed "amazing growth" (Parsons 1952). Growth of roach (*Leuciscus rutilus*) and European yellow perch (*Perca fluviatilus*) improved in acidified Swedish lakes (Almer 1972). Jenson and Snekvik (1972) reported that, prior to extinction, salmonid populations in acidifying Norwegian lakes were characterized by recruitment failures and increased growth rates of individuals. Schofield (1975) recorded an increased growth rate of brook trout (*Salvelinus fontinalis*) in an acidified lake in the Adirondack Mountains.

LAKE MORPHOMETRY

To clarify further the factors affecting the growth of rock bass in the La Cloche lakes, the relationship of growth rate to the morphoedaphic index and its components was investigated. It was felt that if growth rate were primarily a function of abundance in relation to carrying capacity, the inclusion of an index of lake productivity would increase the amount of explained variation in the growth rate of the La Cloche rock bass. Accordingly the relationship between each of the four descriptive lake statistics and the mean age of rock bass at 110 mm was tested by least-squares regression for 24 populations (two populations were not included: Great Mountain Lake and George Lake in 1966). To obtain the best fit, logarithmic transformations were used for each variate, except pH. The resulting correlation matrix describes the strength of the relationship between each of the five variates (Table 4).

Mean lake depth was the strongest single predictor of growth, accounting for 47% of the variance in the growth statistic. There was also a distinct separation of the data points about the regression line describing the relationship between mean depth and the rock bass growth statistic (Fig. 5). Given lakes of similar mean depth, those of higher levels of acidity tended to contain faster-growing rock bass.

The independent variate accounting for most of the variance in the growth statistic in conjunction with the strongest predictor was lake pH, as determined by stepwise, linear, multiple regression. The addition of lake pH to the regression equation accounted for an additional 32% for a total of 79% of the variance accounted for in the dependent variate (Fig. 6). The morphoedaphic index, although significantly correlated ($P \leq 0.01$) with the growth statistic, was a weaker predictor than its component, mean depth. Total dissolved solids was not significantly correlated with the growth statistic but was with lake pH ($P \leq 0.05$).

Shallow lakes tend to be more productive in terms of fish yield or have a greater carrying capacity than deep lakes. This trend, explicit in Ryder's (1965) morphoedaphic index, is thought to be due to a number of interdependent relationships such as the higher mean temperatures in the epilimnia of shallow lakes (Patalas 1975), the greater variation in the environment provided by shallow lakes (Regier and Henderson 1973), and the proportionately greater bottom area of shallow lakes lying within the euphotic zone (Ryder et al. 1974). Among the La Cloche lakes possessing similar levels of potential productivity, or

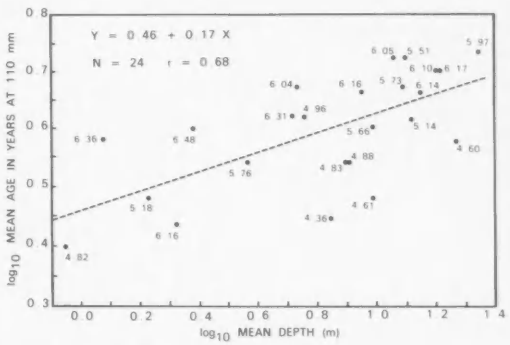

FIG. 5. Relation between lake mean depth and mean age of rock bass at 110 mm. Numerals record the lake pH corresponding to each data point.

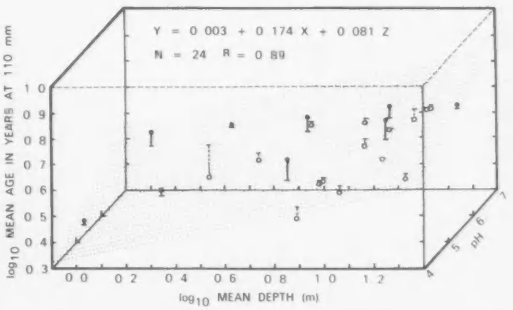

FIG. 6. Mean age of rock bass at 110 mm in relation to mean depth and pH. Points lying above and below the regression plane are shown by closed and open circles, respectively. Vertical lines indicate deviation from the plane.

similar mean depths, those with higher levels of acidity tend to possess faster-growing individuals.

That mean depth was a better predictor of fish growth in the La Cloche lakes than the morphoedaphic index itself can be attributed to the consistently low values of total dissolved solids in La Cloche lakes. Apparently, apart from the airborne acidifying contaminants, the edaphic factor plays a smaller role in productivity in the La Cloche lakes than does the morphometric factor. The relative importance of these two factors, typical of low diversity or oligotrophic environments (Odum 1971), was likely emphasized by the removal of nutrients from the lake waters during acidification.

Almer (1972) and Almer et al. (1974) found that the acidified Swedish lakes usually were waters of great clarity and had low concentrations of nitrogen and phosphorus. They reported that in acidified lakes, the decomposition of organic substances is diminished, fixation of nitrogen is inhibited, phosphates and humic substances are precipitated, and algal content is reduced. Similar events have occurred in the La Cloche lakes as evidenced by the positive relationship of total dissolved solids to lake pH. However, for lakes characterized by a greater variation in trophic status than the La Cloche lakes, the morphoedaphic index would be the more appropriate statistic for monitoring changes in fish abundance reflected in the growth rate.

Conclusions

1. Mean lake depth was the best single predictor of the growth rate of rock bass, accounting for 47% of the variance in the growth statistic, mean age at 110 mm; 2. the growth of rock bass tended to be faster in lakes of greater acidity as a result of reduced population densities with lake pH accounting for an additional 32% of the variance, for a total of 79%; 3. for lakes undergoing stress, the growth rate of fishes, taken in conjunction with the morphoedaphic index, appears to have considerable diagnostic value.

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Long-Term Toxic Effects of DDT Food and Water Exposure on Fathead Minnows (*Pimephales promelas*)

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JARVINEN, A. W., M. J. HOFFMAN, AND T. W. THORSLUND. 1977. Long-term toxic effects of DDT food and water exposure on fathead minnows (*Pimephales promelas*). J. Fish Res. Board Can. 34: 2089–2103.

Fathead minnows (*Pimephales promelas*) were exposed during a partial chronic toxicity test to two DDT concentrations in the water, one in the diet, and combinations of water and diet for 266 days through a reproductive period of their life cycle. Tissue residue analyses were performed at preset intervals throughout the exposure and also on embryos, larvae at hatch, and 30- and 60-day progeny. The contribution of DDT from each source was monitored with gas chromatography and liquid-scintillation techniques. The fish were fed a diet of clams that had accumulated ^{14}C-DDT when exposed at a DDT water concentration similar to that in the high fish exposure. Higher total DDT tissue residues were accumulated from the water than diet. Residues contributed by dietary DDT were additive to those from the water. Mean concentration factors were 1.2 for the diet and 100,000 for the water. Fish exposed to DDT in both water and diet had higher mortality rates than those exposed to only one or the other of these sources. DDT in the diet significantly reduced the probability of survival of the test fish ($P = 0.025$). Estimated maximum acceptable toxicant concentrations for DDT are 0.9 μg/ℓ for fish exposed to DDT in the water only and 0.4 μg/ℓ for fish exposed to DDT in both water and diet. Embryo residue levels and larval mortality rates were about twice as great for those from parent fish that had been exposed to DDT in both the water and diet as for those from parent fish exposed to only DDT in the water.

Key words: toxicity, DDT, pesticides, food chain, ^{14}carbon, bioassay, minnows, clams, aquatic animals

JARVINEN, A. W., M. J. HOFFMAN, AND T. W. THORSLUND. 1977. Long-term toxic effects of DDT food and water exposure on fathead minnows (*Pimephales promelas*). J. Fish. Res. Board Can. 34: 2089–2103.

Lors d'un essai de toxicité chronique partielle, nous avons exposé des têtes-de-boule (*Pimephales promelas*) à deux concentrations de DDT dans l'eau, une dans la nourriture et dans des combinaisons d'eau et de nourriture durant 266 jours comprenant la période reproductrice de leur cycle biologique. Nous avons effectué des analyses de résidus dans les tissus à intervalles prédéterminés durant toute l'exposition, de même que sur des embryons, des larves à l'éclosion et des alevins de 30 et 60 jours. Nous avons suivi la contribution de chaque source en DDT par chromatographie en phase gazeuse et techniques de scintillation liquide. On a donné aux poissons un régime de coques qui avaient accumulé de ^{14}C-DDT lors d'une exposition à une concentration de DDT dans l'eau semblable à celle utilisées dans la haute exposition des poissons. Il y a plus grande accumulation totale de résidus tissulaires de DDT en provenance de l'eau qu'en provenance de la nourriture. Les résidus provenant du DDT alimentaire s'additionnent à ceux de l'eau. Les indices de concentration moyenne sont de 1.2 pour la nourriture et de 100 000 pour l'eau. Les poissons exposés au DDT à la fois dans l'eau et dans la nourriture ont des taux de mortalité plus élevés que ceux exposés à l'une ou l'autre de ces sources. Le DDT dans la nourriture diminue de façon significative la probabilité de survie des poissons d'essais ($P = 0.025$). Les concentrations acceptables maximales estimées de DDT sont de 0.9 μg/ℓ pour des poissons exposés au DDT dans l'eau seulement ou de 0.4 μg/ℓ pour des poissons exposés au DDT à la fois dans l'eau et dans le régime. Les niveaux de résidus dans les embryons et les taux de mortalité des larves sont environ du double chez ceux provenant de parents qui avaient été exposés au DDT à la fois dans l'eau et dans la nourriture comparativement à ceux dont les parents avaient été exposés au DDT dans l'eau seulement.

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THERE are many opinions among aquatic researchers about the importance of water concentration and food chain as sources for biological magnification of pesticides in the aquatic environment. Some authors suggest the food chain as the major source (Macek and Korn 1970); Harrison et al. 1970; Johnson et al. 1971; Eberhardt et al. 1971), whereas others (Reinert 1967, 1970; Chadwick and Brocksen 1969; Grzenda et al. 1970; Murphy 1971; Hamelink et al. 1971; Epifanio 1973) stress the water concentration. No data are available regarding those situations where both sources are involved at threshold levels of chronic toxicity.

Current pesticide standards are based upon water concentrations alone. We must also know what effect the presence of pesticide-laden food-chain organisms has on aquatic life so that accurate pesticide standards can be developed. The following study was initiated in response to these problems. Our objectives were to determine whether DDT accumulation in fathead minnows (*Pimephales promelas*) is more affected by a food or water source, to determine whether persistent pesticide exposure through both food and water is more toxic (or creates higher residues) than exposure through only one or the other of these routes, to estimate a partial chronic maximum acceptable toxicant concentration for DDT, and to determine DDT concentration factors for fathead minows and freshwater clams used as the food.

Materials and Methods

PHYSICAL CONDITIONS

All fathead minnow and clam exposures were conducted at the Environmental Research Laboratory-Duluth, Duluth, Minnesota.

Fathead minnow exposure — The basic design of the partial chronic exposure followed the recommended procedures set forth by the National Water Quality Laboratory Committee on Aquatic Bioassays (Jarvinen et al. 1976). A proportional diluter (Mount and Brungs 1967) was modified to deliver two test concentrations and a control with the low test concentration one quarter that of the high concentration. The toxicant was introduced by a 50-ml injector syringe with a Teflon® needle from an acetone stock solution. The highest level of acetone ever reached, within any 24-h period, in the high concentration test chamber was 5 mg/ℓ. Nominal DDT test concentrations, selected on the basis of acute and preliminary partial chronic data, were 2.0 and 0.5 μg/ℓ, respectively.

The test water was sand-filtered Lake Superior water sterilized with ultraviolet light and warmed to approximately 25°C by a coiled stainless steel heat exchanger located in a stainless steel headbox. A thermoregulator relay system (Syrett and Dawson 1972) activated tandem solenoid valves that controlled the flow of hot water through the heat exchanger.

The test chambers used for adult exposures measured 91 × 30 × 30 cm and held a water volume of 55 ℓ. Approximately 3 mo after the start of the test the adult tank was separated into two sections by stainless steel screen. Two 30.5 × 13.5 × 31.5-cm larval chambers were placed in the back section of each adult chamber, and flow rates were adjusted to provide 250 ml of test water to each larval chamber and 500 ml to the adult section. The flow of water to the test chambers was adjusted to maintain dissolved oxygen levels at >65% saturation and to provide a 99% replacement time of the test water in each test chamber within 10 h as determined from Sprague (1969).

Chambers were siphoned daily 1–2 h after feeding to remove leftover food and were brushed and siphoned weekly.

The photoperiod followed the normal daylight hours of Evansville, Indiana, except that it was necessary to extend the peak photoperiod of 15 h 45 min for approximately 6 wk to insure enough larvae for gas-chromatographic and liquid-scintillation analyses of tissue residues. Daytime light intensity varied from 269 to 441 lx in the adult chambers.

Clam exposure — Clams were exposed to ^{14}C-DDT in a flow-through system as described by Jarvinen et al. 1976. The toxicant was introduced into the toxicant chamber by syringe for a nominal DDT concentration of 2.0 μg/ℓ.

BIOLOGICAL CONDITIONS

Fish — On September 13, 1972, one hundred 45(±3)-day-old fathead minnows were randomly put in each of 12 test chambers. The 12 chambers were used to expose the fish through a reproductive period of their life cycle to duplicates of (1) a control, (2) DDT-exposed food, but no DDT in the water, (3) unexposed food, but 0.5 μg/ℓ DDT water exposure, (4) DDT-exposed food and 0.5 μg/ℓ DDT water exposure, (5) unexposed food, but 2.0 μg/ℓ DDT water exposure, and (6) DDT-exposed food and 2.0 μg/ℓ DDT water exposure. The fathead minnows were fed chopped and oven-dried clam tissue that was either clean or contaminated by ^{14}C-DDT.

Fish were sampled for total body tissue residues of DDT and percent lipids at 7, 14, 28, 56, 112, 224, and terminally at 266 days' exposure. The fish were not fed for 24 h before samples were taken. Ten fish were sampled from each test chamber after 7 days of exposure and five fish were sampled from each chamber thereafter, until the end of the exposure period when all remaining fish except those used for an elimination study were sampled. Samples were placed in preweighed glass vials, reweighed, and frozen at −8°C until analyzed. Results were determined on a whole-body wet-weight and lipid basis.

During the spawning period embryos in excess of the 50 required for hatchability studies were placed

in preweighed glass vials, reweighed, preserved with petroleum ether to prevent dehydration, and stored in a freezer at −8°C. Individual test-chamber samples were composited to provide a minimum of four 0.3-g (about 300 embryos) samples for residue analysis.

Larvae at hatch were transferred in groups of 40 to larval chambers for 30- and 60-day growth, mortality, and residue studies. The fish were photographed at 30 and 60 days by the method of Martin (1967) as modified by McKim and Benoit (1971) for growth determination. Tissue residues were analysed for two 30- and one 60-day samples for each of the 12 test chambers.

Larvae at hatch not saved for 30- and 60-day studies were weighed and grouped by the same method used for embryo-residue samples to provide a minimum of two 0.3-g (about 600 larvae) samples per test chamber for residue analysis. Residue analysis of larvae at hatch and embryos was performed on a whole-body wet-weight basis.

To simplify the presentation of results test treatments are coded as follows:

DDT exposure	Coding
Clean water, clean food (Control)	C
Clean water, DDT food	F
0.5 μg/ℓ DDT water, clean food	0.5 W
0.5 μg/ℓ DDT water, DDT food	0.5 W + F
2.0 μg/ℓ DDT water, clean food	2.0 W
2.0 μg/ℓ DDT water, DDT food	2.0 W + F

Results from duplicate chambers were combined, and the data are expressed as the mean ± SE unless otherwise indicated.

Clams — Chopped, oven-dried clam tissue was used as the food source. Five species of clams were collected from the Eau Claire River in Wisconsin: *Lampsilis siliquoidea*, *L. ventricosa*, *Lasmigona costata*, *Fusconaia flava*, and *Ligumia recta*. Before clams were exposed to DDT they were held for a minimum of 8 wk in a fiberglass tank through which lake water flowed. Each of four groups of clams was exposed to ^{14}C-DDT for 8 wk. The exposure time was chosen because preliminary studies indicated that an 8-wk exposure was necessary for the clams to achieve an equilibrium with an exposure concentration of 2.0 μg/ℓ DDT (the same DDT concentration as in the higher fish water exposure).

Clams were placed in the exposure chambers and slowly acclimated to 20°C. Fifty clams were used in each duplicate chamber per exposure, and 100 were held in a fiberglass control chamber through which lake water flowed from the same headbox as the exposure system. The control chamber had a volume of 170 ℓ and a water flow rate to provide 99% replacement in 6 h. Dissolved oxygen concentrations never dropped below 80% saturation.

The clams were fed daily with a commercial fish fry food and plankton. The flow-through apparatus was monitored daily, the chambers were siphoned every other day, and the sides of the chambers were scrubbed whenever algal or fungal growth became excessive.

After completion of an 8-wk exposure, the soft parts of the clams were removed, chopped in a blender, and oven-dried for 1½–2 h at 100°C. The dried clam meat was supplemented with a vitamin and mineral mix. A list of ingredients for the mix was obtained from the Fish-Pesticide Research Laboratory, Columbia, Missouri (Paul Mehrle, Fish-Pesticide Research Laboratory, Columbia, Missouri 65201, personal communication). The prepared food supply was kept frozen, and a small portion was removed daily for feeding.

Chemical Conditions

Fish — Water temperatures were maintained between 24.0 and 25.5°C and were checked daily in all test chambers. Routine water chemistries were determined weekly by the methods described by APHA et al. (1971). Dissolved oxygen levels were never lower than 5.4 mg/ℓ nor higher than 8.2 mg/ℓ. Mean total hardness, acidity, and alkalinity were 43.9, 2.8, and 42.5 mg/ℓ, respectively; pH was between 7.2 and 7.8. DDT stock solutions were prepared with DDT, Technical grade (*p,p'*-isomer 77%, Nutritional Biochemicals Corporation, Cleveland, Ohio) and DDT concentrations in the water were measured once a week. In each sample set, analyses were made on a duplicate and a spiked sample of control water. DDT was extracted from the test water with petroleum ether and analyzed by gas chromatography. Percent recovery from the spiked control water samples ranged from 38 to 115%; mean recovery was 86.9 ± 3.4% ($n = 39$).

Determined DDT water concentrations in the test exposures are presented in Table 1.

Clams — The *p,p'* DDT ring-UL-^{14}C (Mallinckrodt/Nuclear, St. Louis, Missouri) in benzene with a specific activity of 3.85 mCi/mM was procured in 50-μCi lots. The benzene was evaporated under a stream of nitrogen, and the *p,p'* DDT ring-UL-^{14}C was redissolved in 50 ml of acetone. The ^{14}C-DDT stock solutions were prepared by dissolving a calculated gram weight of DDT (Tech.) in acetone to which 45 ml

Table 1. DDT water concentrations in exposure chambers. SD in parentheses.

Nominal DDT water concn (μg/ℓ)	Measured concn (μg/ℓ)		
	N	Mean	Range
2.0 W	41	1.53 (0.35)	0.82[a]–2.30
2.0 W + F[b]	41	1.48 (0.30)	0.82[a]–2.00
0.5 W	41	0.35 (0.11)	0.16[a]–0.70
0.5 W + F[b]	41	0.37 (0.11)	0.19[a]–0.79
F[b]	41	0.01 (0.03)[c]	0.00–0.10[c]
C	41	0.00 (0.01)	0.00–0.06[d]

[a]Syringe injector malfunction (leakage).
[b]Fed clam tissue with ^{14}C-DDT (45.6 mg/kg).
[c]Leaching from ^{14}C-DDT food.
[d]Possible contamination from wrong food; occurred only once during exposure.

of the ^{14}C-labeled DDT in acetone was added. Total volume was brought to 250 ml with acetone and thoroughly mixed. Only enough ^{14}C-labeled DDT to give readable scintillation counts was combined with the unlabeled DDT. This procedure insured that the same stock of unlabeled material was the major source of DDT used in all fish and clam exposures. An additional benefit was a reduction in the amount of labeled DDT that would have been needed in the flow-through exposures. The clams were exposed to 2.0 $\mu g/\ell$ ^{14}C-DDT nominal concentration. The ^{14}C-DDT water concentrations ranged from 1.05 to 2.60 $\mu g/\ell$, with a mean concentration of 1.81 $\pm$ 0.13 ($n = 14$). Percent recovery of spiked control water samples ranged from 80 to 121%; mean recovery was 102.6 $\pm$ 8.5% ($n = 5$). Water analysis for DDT was performed by the same method as for the fathead minnow exposure.

Residue Analysis

Gas chromatography — An Eberbach Semi-Micro or Micro Container explosion-proof blender (depending upon sample weight) was used for extraction of adult fish and 30- and 60-day progeny. Anhydrous Na_2SO_4 was added to dry the tissues and insure homogenization. Samples were blended with petroleum ether for 2 min at high speed. The solvent extract was decanted onto an anhydrous Na_2SO_4 column and collected in a tared beaker for lipid determination. Each sample was extracted 3 times. The solvents were evaporated, and lipid weight was determined. The lipids were redissolved in petroleum ether and then cleaned up on a 20-g Florisil column. Samples were eluted with 200 ml of 6% ethyl ether–petroleum ether as described by the U.S. Department of Health, Education, and Welfare (1971). Samples were concentrated to a volume of < 10 ml on a steam bath. Analysis was completed by gas chromatography. Peak heights were measured individually for DDT, DDE, and TDE. DDT and metabolites were then summed to obtain the total DDT present. DDT, DDE, and TDE are expressed as the sum of the *o,p'*- and *p,p'*-fractions found in the analyses.

Samples of embryos and larvae at hatch were homogenized in 10 ml of petroleum ether in a glass tissue-grinding tube by using a Teflon pestle. Samples were extracted 5–8 times depending upon total sample weight and were concentrated as previously described for the adult fish. No cleanup was necessary and no lipid analysis was performed. Residue analysis was performed as for adult fish. Residue analysis on the clams was conducted on duplicate 1-g samples of tissue from both control and ^{14}C-DDT-exposed clams before addition of the vitamin–mineral supplement. The samples were extracted in a blender with 35% water–acetonitrile and then partitioned with 100 ml of petroleum ether. Cleanup was performed as described for the adult fish.

Liquid scintillation — We used a Packard Tri-Carb liquid scintillation spectrometer to determine DDT residues attributed to the food for all samples. Samples were prepared from the portion of the extracted tissue residues not used in gas-chromatographic analysis. The samples were evaporated to 0.2 ml in glass concentrator tubes and were then transferred to glass scintillation vials with four 1-ml washings of toluene. Fifteen millilitres of Instagel (Packard Instrument Company, Inc., Downers Grove, Illinois) (scintillation cocktail) were added to each vial. The vials were kept in the scintillation counter overnight to allow them to cool and dechemiluminesce before analysis.

A correlation was made between the gas chromatograms and scintillation counts through a series of dilutions of the ^{14}C-DDT stock solution. An average count per minute per microgram of DDT was then calculated and used for the determination of the micrograms of DDT attributed to the contaminated food source. Before final calculations were made, individual sample counts per minute were corrected for background radiation and sample volumes removed for gas-chromatographic analysis. Final results were calculated on a whole-body wet-weight basis.

Statistics

Mortality results were analyzed by calculation of the accumulative probability of fish survival over the different sample periods during the toxicity test as described by Jarvinen et al. (1976).

All survival and egg-hatchability data were transformed to arcsin $\sqrt{\%}$. Two-way analysis of variance was applied to all survival, embryo-hatchability, percent lipid, and 30- and 60-day progeny growth data to determine the effect of exposure to DDT in food or water. Dunnett's procedure (Steel and Torrie 1960) was used for comparison of treatment means with control means. We used nonlinear least square estimation (Dixon 1973) to determine values for the food and water parameters that affected adult survival.

Results

Adult Fish

Concentrations of total DDT (DDT + DDE + TDE) in the fish reached an equilibrium with the concentration in the diet within 28–56 days (Fig. 1). The additive residue effect of dietary total DDT when compared with residues from a water source only is also demonstrated (Fig. 1, 2). In general, total residues in fish exposed to DDT in food (F) and 0.5 $\mu g/\ell$ in water (0.5 W) peaked by 56 days, whereas concentrations in fish exposed to the other combinations of DDT in food and water peaked by 112 days. An equilibrium may have been reached at 0.5 W within 56 days. In general, residue levels decreased rapidly during the spawning period (112–224 days) and then increased after termination of spawning activity.

After 14 days of exposure, residues of DDT in fish exposed to DDT in both the water and

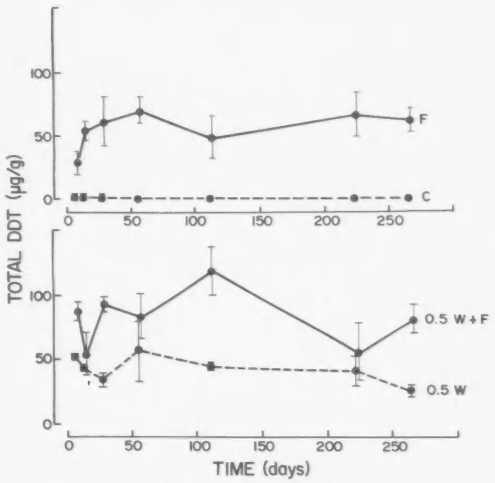

FIG. 1. Total DDT residues (micrograms per gram) in the controls and fish exposed to DDT in the food (*upper*) and in fish exposed to DDT in the water (0.5 $\mu g/\ell$) or in combination of food and water (*lower*). (Vertical lines indicate standard error.)

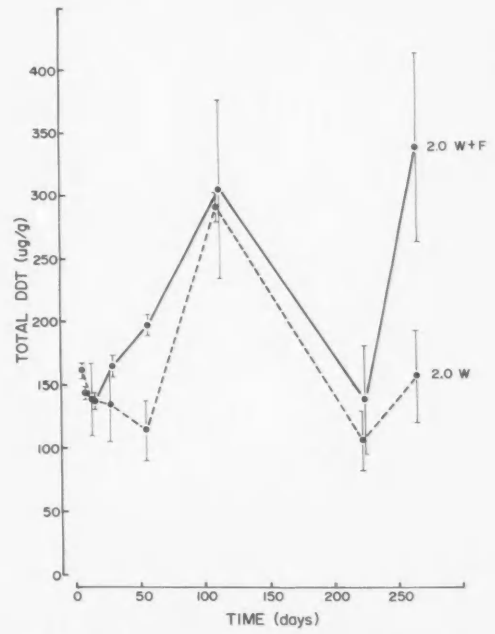

FIG. 2. Total DDT residues (micrograms per gram) in fish exposed to DDT in the water (2.0 $\mu g/\ell$) or in combination of food and water. (Vertical lines indicate standard error.)

food were generally greater than for those exposed to the insecticide in the water only.

DDE was the principal DDT residue found after 14 days of exposure, an indication that DDT was rapidly metabolized (Table 2). Lack of TDE in the residues of fish fed DDT-exposed clam tissue in the 7–56-day samples was caused by failure of the gas-chromatographic column to differentiate *p,p'*-TDE from *o,p'*-DDT. Column changes permitted differentiation for the 112 day and later samples. In general, DDT levels decreased rapidly after 14 days. The clams metabolized very little DDT to DDE; the principal metabolite was TDE (Table 3).

The ^{14}C-DDT content of the clam meat from the four exposures averaged 45.6 ± 3.8 µg/g ($n = 4$) as determined by gas chromatography. This value indicates a concentration factor of 25,000 based upon the average concentration of DDT measured in the water. Total DDT in the clam tissue consisted of 68% DDT, 1% DDE, and 31% TDE.

Use of ^{14}C-labeled DDT in the contaminated food allowed us to use liquid-scintillation analysis (LS) to separate the DDT contributed by the food from the total amount of DDT as determined by gas-chromatographic analysis (GC). Gas-chromatographic and liquid-scintillation results were not identical as they should have been for the fish exposed to ^{14}C-DDT-contaminated food only. With $n = 19$, the mean liquid-scintillation value was 110% of the gas-chromatographic value with SD = 28.4%. To correct for this difference we adjusted LS values to GC values by the following formula: adjusted LS value = LS value obtained/ratio LS value to GC value for F-exposed fish. These data are presented in Table 4 and are expressed as a percentage of the total residues as determined by gas-chromatographic analysis. DDT in the food apparently had a maximum input within 28–56 days. The relative amount contributed by the DDT-contaminated food was about 60% of the total DDT residues in fish exposed at 0.5 W + F and about 30% in fish exposed at 2.0 W + F.

Mean calculated concentration of total DDT from food and water was 1.2 ± 0.1 times for the DDT from the food ($n = 57$) and 99,000 ± 7000 times for DDT from the water ($n = 39$, 0.5 W and 2.0 W exposures combined).

Lipid percentages were also determined for the test fish. No significant difference ($P = 0.05$) was found in the percentage of lipids between fish exposed to DDT in the water only and fish exposed to DDT in the water and fed DDT-contaminated food.

Fish fed a DDT-contaminated diet were less

TABLE 2. DDT, DDE, and TDE residues (micrograms per gram) in fish exposed to various test conditions (duplicate samples combined; each sample is a composite of 5–10 fish). SE is in parentheses.

Nominal DDT water concn (μg/ℓ)	Days of exposure																					
	7			14			28			56			112			224			266		
	DDT	DDE	TDE	DDT	DDE	TDE	DDT	DDE	TDE	DDT	DDE	TDE	DDT	DDE	TDE	DDT	DDE	TDE	DDT	DDE	TDE
C	1.0 (0.5)	0.7 (0.2)	0.1 (0.1)	1.0 (0.2)	1.0 (0.0)	0.0	0.6 (0.1)	1.0 (0.5)	0.0	0.2 (0.0)	0.3 (0.1)	0.0	0.1 (0.0)	0.2 (0.0)	0.1 (0.0)	0.1 (0.0)	0.1 (0.0)	0.0	0.0	0.1 (0.2)	0.0
F	16.2 (5.7)	12.0 (3.4)	0.0	28.2 (3.2)	24.1 (4.0)	1.0 (0.1)	23.8 (4.7)	35.3 (12.9)	1.1 (1.1)	18.5 (1.5)	49.4 (8.6)	1.0 (0.2)	7.0 (1.9)	38.5 (13.9)	15.0 (3.8)	5.2 (1.7)	47.5 (11.7)	12.5 (3.8)	5.0 (1.0)	33.3 (4.6)	22.4 (3.9)
0.5 W	26.1 (0.6)	24.9 (0.8)	0.0	18.4 (5.4)	24.3 (4.7)	0.0	7.2 (2.0)	25.5 (2.9)	0.7 (0.0)	13.2 (6.8)	18.8 (7.4)	0.6 (0.6)	6.1 (0.3)	36.8 (1.0)	3.7 (0.7)	3.5 (1.0)	36.7 (11.4)	0.8 (0.2)	2.7 (0.7)	21.7 (3.2)	1.0 (0.2)
0.5 W + F	47.4 (5.4)	39.0 (1.7)	0.0	22.5 (10.6)	29.5 (5.2)	1.4 (0.5)	35.9 (7.4)	53.7 (2.0)	3.1 (0.6)	17.7 (3.4)	63.3 (14.4)	1.4 (0.5)	14.2 (5.1)	120.2 (33.8)	20.4 (5.2)	4.9 (2.1)	41.7 (17.2)	7.9 (3.7)	7.5 (1.5)	54.1 (7.0)	18.2 (2.9)
2.0 W	94.2 (5.3)	66.8 (0.9)	0.0	48.5 (8.8)	88.3 (19.6)	1.4 (0.3)	37.0 (13.9)	92.6 (14.1)	4.0 (0.9)	25.1 (8.2)	86.6 (14.8)	1.7 (0.3)	58.0 (0.6)	230.9 (34.9)	19.3 (1.6)	9.5 (2.5)	93.6 (20.7)	9.1 (1.0)	15.8 (4.6)	136.9 (31.4)	3.8 (0.8)
2.0 W + F	92.8 (4.9)	50.3 (0.0)	0.0	77.8 (23.2)	54.7 (28.3)	3.8 (0.8)	43.3 (3.7)	113.4 (12.1)	7.4 (0.2)	44.6 (0.5)	149.2 (8.4)	3.1 (0.9)	49.8 (19.0)	246.6 (67.8)	52.2 (13.5)	18.0 (7.3)	113.3 (32.8)	6.2 (3.3)	28.5 (8.1)	291.7 (62.0)	17.1 (5.1)

likely to survive than those fed clean food (Fig. 3). To test whether the addition of DDT-contaminated food altered the probability of survival, a two-way analysis of variance was run on the survival data. The hypothesis that the presence of DDT in the food does not change survival was rejected at the $P = 0.025$ level. The accumulative reduction in the probability of survival (Fig. 3) indicates two definite periods of high mortality, the juvenile stage during the first 28 days of exposure (test started with about 45-day-old fish; therefore, the fish were 73 days old at 28 days exposure) and during spawning between 112 and 224 days of exposure. Mortality was greater in fish fed DDT-contaminated food and remained high through 56 days' exposure. The death rate was also greater for fish fed DDT-contaminated food during the spawning period. Fish that died during the spawning period were predominantly adult males (about 83% males, 17% females). Residue levels in these fish were only about 55% of those in live sampled fish. Lipid percentages however, were very low (mean 1.07%, $n = 23$, all samples combined) when compared with the live fish (mean 3.1%, $n = 12$, all samples combined). The percentage of residue levels attributed to DDT in the food among fish that died

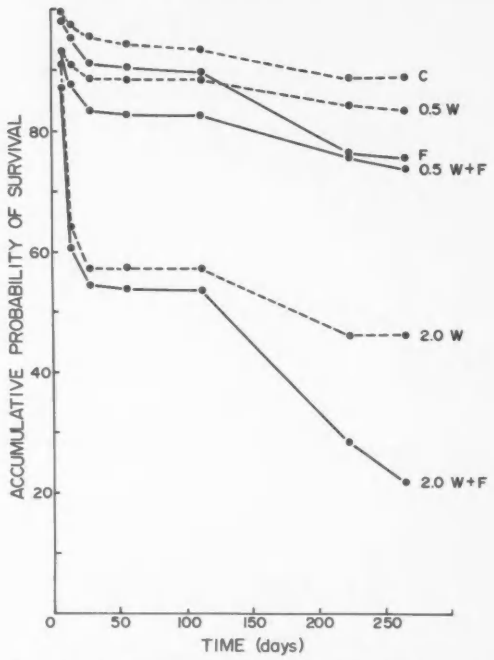

FIG. 3. Accumulative probability of survival for fish exposed to DDT.

TABLE 3. DDT, DDE, and TDE residues (micrograms per gram) in the fish food (oven-dried weight; 1½–2 h; 110°C). —, not detectable.

Exposures	Time period when used as food (days)	DDT	DDE	TDE	Time of year collected	Time of year exposed to ^{14}C-DDT
		^{14}C-DDT-exposed clams				
1	0–112	42.6	0.6	14.3	Fall	Spring (late)
2	112–224	38.2	0.5	2.7	Spring	Fall
3	112–224	31.2	0.2	9.7	Fall	Winter
4	224–266	11.0	0.2	29.0	Fall	Spring (early)
		Clean clams				
1	0–112	0.7	—	—	Fall	Spring (late)
2	112–224	—	—	—	Spring	Fall
3	112–224	—	—	—	Fall	Winter
4	224–266	—	—	—	Fall	Spring (early)

TABLE 4. Percentage of total DDT caused by the ^{14}C-DDT food source (duplicate samples combined; liquid-scintillation values shown are adjusted). SE in parentheses; *n = 1; **n = 4.

Nominal DDT water concn (μg/ℓ)	Days of exposure							Mean for entire period
	7 (n = 2)	14 (n = 2)	28 (n = 2)	56 (n = 2)	112 (n = 2)	244 (n = 4)	266 (n = 6)	
F	100.0 (1.4)	100.0*	100.0 (2.8)	100.0 (13.4)	100.0(6.2)	100.0 (2.7)	100.0 (3.0)	100.0 (1.6) (n = 19)
0.5 W + F	22.5 (0.0)	46.9 (19.4)	76.4 (3.4)	81.7 (4.2)	55.4 (1.4)	72.2 (5.4)	64.7 (1.4)	62.1 (4.2) (n = 20)
2.0 W + F	4.8 (2.1)	28.1 (15.7)	38.2 (3.4)	50.7 (5.6)	33.9 (0.9)	28.7 (2.3)	17.6** (1.2)	27.6 (3.4) (n = 18)

TABLE 5. Hatchability; mean DDT, DDE, TDE, and total DDT residues (micrograms per gram); and percent total DDT contributed by the contaminated food in embryos from fish exposed to various test conditions (duplicate samples combined). N = number of spawnings; SE in parentheses; —, not detectable; LS = liquid scintillation.

Nominal DDT water concn (μg/ℓ)	N	% hatch	No. of samples	DDT	DDE	TDE	Total DDT	% total DDT caused by DDT in the food, LS adjusted
C	62	88.9	15	0.1 (0.0)	0.4 (0.0)	—	0.5 (0.0)	—
F	44	85.7	12	1.6 (0.1)	7.8 (0.9)	2.8 (0.2)	12.2 (1.1)	100 (6.2)
0.5 W	51	84.4	16	0.8 (0.1)	5.7 (0.6)	0.3 (0.0)	6.8 (0.7)	—
0.5 W + F	55	89.2	15	2.0 (0.4)	13.7 (2.2)	3.3 (0.6)	19.0 (3.1)	68 (1.8)
2.0 W	98	74.1[a]	23	3.8 (0.5)	19.6 (1.7)	0.6 (0.1)	24.0 (2.0)	—
2.0 W + F	51	78.3	18	5.3 (0.4)	31.3 (2.9)	4.2 (0.6)	40.8 (3.8)	28 (0.5)

[a]Significantly different from the control (P = 0.05). Dunnett's procedure (Steel and Torrie 1960).

during spawning was 100% of the DDT at the F exposure, 70% at the 0.5 W + F exposure, and 23% at the 2.0 W + F exposure. These percentages are similar to those found in the similarly exposed live fish.

EMBRYOS

Presence of DDT in the parents' food did not significantly alter hatchability, whereas DDT in the water did (Table 5). The reduction in hatchability, however, was significant only for embryos from parent fish that were subject to the 2.0 W exposure. The percentages of total DDT contributed by contaminated food fed to the parent fish are similar to those in residues from the adult fish. Residues from DDT-contaminated food ap-

TABLE 6. Mean DDT, DDE, TDE, and total DDT residues (micrograms per gram); and percent total DDT contributed by the contaminated food in larvae at hatch from fish exposed to various test conditions (duplicate samples combined). SE in parentheses; —, not detectable; LS = liquid scintillation.

Nominal DDT water concn ($\mu g/\ell$)	No. of samples	DDT	DDE	TDE	Total DDT	% total DDT caused by DDT in the food, LS adjusted
C	6	—	0.2 (0.0)	—	0.2 (0.0)	—
F	4	3.0 (0.3)	18.6 (2.6)	4.8 (0.2)	26.4 (2.9)	100 (0.8)
0.5 W	4	3.8 (0.3)	18.9 (2.3)	1.3 (0.0)	24.0 (2.2)	—
0.5 W + F	6	5.7 (0.5)	32.8 (4.5)	5.0 (0.6)	43.5 (4.3)	60 (1.9)
2.0 W	9	19.9 (0.9)	66.5 (5.3)	1.5 (0.1)	87.9 (5.9)	—
2.0 W + F	5	20.1 (2.0)	73.0 (15.4)	2.9 (0.7)	96.0 (17.8)	20 (0.6)

pear to be additive to those attributed to water exposure.

DDE was the primary metabolite. Some DDT from the water was metabolized to TDE, but the amount is only about 10% of that attributed to the food source. There is increased TDE with higher water exposure for the embryos from the water plus food-exposed fish.

LARVAE AT HATCH

When compared with embryo residues, residues in larvae at hatch from parent fish fed dietary DDT are about 2 times higher, whereas those from parent fish exposed to DDT only in the water are about 3.6 times higher (Table 6). Part of this difference is explained by the reduction in weight of the larvae at hatch to almost one half that of the embryos. Therefore, if nearly all the residue were contained within the developing larvae, the calculated residue level would automatically be 2 times greater at hatch when the embryo membrane and surrounding fluid is lost. The percentage of total DDT resulting from DDT-contaminated food was about 8% lower for larvae at hatch than for embryos. Larvae were removed for analysis daily, so it is possible that some may have been exposed to DDT in the water for a maximum of 24 h before being removed.

DDE again is the principal constituent. High TDE levels in larvae from food-exposed parents are attributed to the food source.

PROGENCY AT 30 AND 60 DAYS

Residues of DDT in progeny are slightly higher at 60 than at 30 days, except for fish at the F exposure, where they are lower (Table 7). The percentage of total DDT contributed by the food source is only 8% higher at 60 days than that at 30 days. Progeny from fish exposed to 2.0 W hatched and raised in control water for 30 days contained only 0.5 $\mu g/g$ total DDT in their tissues. Progeny from fish exposed to 2.0 W + F, also hatched and raised in control water and fed clean food for 30 days, had residues of only 0.2 $\mu g/g$ total DDT. As determined by liquid-scintillation analysis, none of this DDT could be traced to the intake of DDT-contaminated food by parent fish. However, progeny from the same parent fish that were hatched and raised in clean water but fed DDT contaminated food for 30 days had total DDT residues of 31.6 $\mu g/g$, of which 93% could be attributed to the food.

In general, DDT residues decreased at most exposures between 30 and 60 days, whereas DDE residues increased. TDE residues also showed a slight increase between 30 and 60 days, except at the 0.5 W + F exposure, where there was a 38% increase.

DDT apparently did not affect the growth of the 30- and 60-day progeny. Two-way analysis of variance on growth data indicated that there was no significant growth difference ($P = 0.05$) between the progeny exposed to DDT in the food or water.

All larvae died within 5 days of hatch at the 2.0 W or 2.0 W + F exposures. Control larvae, in groups of 40 each ($n = 4$), transferred to these concentrations also died within 5 days. A significant effect ($P = 0.05$) on survival was observed for DDT water exposure at 30 and 60 days, but not for food exposure. Use of Dunnett's procedure (Steel and Torrie 1960) indicated that the exposures significantly different from the controls were those at 2.0 W and 2.0 W + F and those from parent fish exposed to 2.0 W + F, whose progeny were hatched and raised in control water and fed clean food for 30 days (Table 8). This latter group experienced a twofold higher death rate than progeny from parent fish exposed at 2.0 W that were hatched and raised in control water and fed clean food. In this higher mortality group, progeny in two groups (40 fish each) ex-

TABLE 7. Mean DDT, DDE, TDE, and total DDT residues (micrograms per gram); and percent total DDT contributed by the contaminated food in 30- and 60-day-old progeny of fish exposed to various test conditions (duplicate samples combined, progeny exposed to same conditions as parent fish unless otherwise indicated). SE in parentheses; —, not detectable; LS = liquid scintillation.

Parent fish nominal DDT water concn ($\mu g/\ell$)	Progeny exposure (days)	No. of samples	DDT	DDE	TDE	Total DDT	% total DDT caused by DDT in the food, LS adjusted
C	30	3	0.1 (0.0)	0.1 (0.0)	0.0 (0.0)	0.2 (0.0)	—
	60	3	0.1 (0.0)	0.2 (0.0)	—	0.3 (0.0)	—
F	30	4	8.1 (2.0)	15.4 (2.9)	12.2 (3.2)	35.7 (1.6)	100 (9.4)
	60	2	4.7 (0.4)	11.7 (0.7)	12.3 (5.8)	28.7 (5.7)	100 (4.1)
0.5 W	30	4	4.6 (0.9)	8.5 (0.9)	0.5 (0.2)	13.6 (1.8)	—
	60	5	6.1 (0.4)	17.3 (3.5)	0.6 (0.2)	24.0 (4.0)	—
0.5 W + F	30	4	12.5 (3.3)	18.6 (3.1)	15.3 (2.4)	46.4 (4.9)	60 (16.3)
	60	2	10.3 (1.0)	26.8 (0.5)	21.1 (0.9)	58.2 (2.4)	68 (0.4)
2.0 W[a]	30	3	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)	0.6 (0.2)	—
2.0 W + F[a]	30	1	0.1	0.1	—	0.2	—
2.0 W + F[b]	30	1	3.2	9.3	19.1	31.6	93

[a]Progeny hatched and raised in control water and fed clean food.
[b]Progeny hatched and raised in control water and fed DDT food.

perienced zero survival and one group had 75% survival. Total DDT residue in the survivors was 0.18 $\mu g/g$. Another group of progeny from the same parent fish similarly hatched and raised in control water but fed DDT-contaminated food had only 19.5% survival with a residue level in survivors of 31.6 $\mu g/g$ total DDT.

Discussion

Our results indicate that uptake from the food is additive to the amount taken up from the water and that equilibrium with the food was reached within 56 days. These findings are similar to those observed by other authors. Grzenda et al. (1970) reported that there was no additional increase in body concentrations in goldfish after dietary DDT exposure (18 $\mu g/g$) for 32 days. Macek et al. (1970) reported an equilibrium with dietary DDT in the liver, brain, and skeletal muscle of rainbow trout after 28 days' exposure. The portion of the total DDT tissue residues in our test that could be directly attributed to the DDT-contaminated diet was about 30% in fish exposed to a concentration of DDT in water equal to that at which the food had been exposed and 60% in fish exposed to a water concentration one fourth that level. This appears to indicate that the main source of DDT uptake is the water. Chadwich and Brocksen (1969) exposed freshwater sculpins to dieldrin in diet and water and observed that a maximum of 16% of the dieldrin was accumulated from the food. They also stated that accumulation from the diet might be expected to be additive, but this was not so in their test. They reported residue levels that were not much different between fish exposed in food and water and in water only, whereas in our test, tissue residues between the two exposures were different after 14 days. Reinert (1967), also in a study with dieldrin, observed that only about one tenth as much of the residues in guppies was accumulated through ingestion of contaminated food when compared with residues in guppies exposed to a water concentration equal to that in which the food was exposed. This test, however, was of a shorter duration (32 days). In our study the highest residue level caused by DDT-contaminated food was one fourth that caused by exposure to DDT in water that was at the same concentration as that to which the food was exposed.

Our mean DDT concentration factor was 1.2 for dietary DDT and about 100,000 for DDT from the water. These values are similar to those observed by other authors. Hunt and Bischoff (1960) observed a concentration factor of 125,000 for brown bullheads from water, and Courtney and Reed (1972) observed a concentration factor of about 100,000 in tissues of golden shiners that were exposed to 0.3 $\mu g/\ell$ DDT in the water for 15 days. Reinert (1967) observed a 0.05 food concentration factor in tissue residues of guppies

TABLE 8. Percentage survival of 30- and 60-day-old progeny of fish exposed to various test conditions (larvae exposed to same DDT exposure as parent fish).

Progeny exposure (days)		Nominal DDT water concn ($\mu g/\ell$)			
		0	0.5	2.0[a]	2.0
		Clean food			
30	Tank A	73.8 ($n=4$)	55.0 ($n=4$)	25.0 ($n=2$)	0 ($n=3$)
	Tank B	55.8 ($n=3$)	65.6 ($n=4$)	62.3 ($n=2$)	0 ($n=3$)
	Grand mean	66.1	60.3	41.0	0[b]
60	Tank A	46.3 ($n=2$)	31.2 ($n=2$)	—[c]	0
	Tank B	45.0 ($n=1$)	66.3 ($n=2$)	—[c]	0
	Grand mean	45.8	49.0	—	0[b]
		DDT-contaminated food			
30	Tank A	73.0 ($n=3$)	57.5 ($n=3$)	23.6 ($n=3$)	0 ($n=2$)
	Tank B	69.9 ($n=3$)	36.7 ($n=3$)	0 ($n=1$)	0 ($n=2$)
	Grand mean	71.2	47.1	18.8[b]	0[b]
60	Tank A	62.5 ($n=1$)	35.0 ($n=1$)	—[c]	0
	Tank B	51.3 ($n=1$)	42.5 ($n=1$)	—[c]	0
	Grand mean	57.0	38.8	—	0[b]

[a]Progeny from parent fish were hatched and raised in clean water and fed clean food.

[b]Values significantly different from the control larvae (dupicate chambers combined), two-way analysis of variance and Dunnett's procedure (Steel and Torrie 1960); $P = 0.05$.

[c]No larvae available for 60-day study.

exposed to a diet of dieldrin-contaminated *Daphnia* (31 $\mu g/g$) for 32 days, but a concentration factor of 1.3 for *Daphnia* fed dieldrin-contaminated algae (71 $\mu g/g$). He stated, however, that the latter concentration factor may have been caused by algae ingested but not yet assimilated, as algal cells were observed in the *Daphnia* digestive tract. Epifano (1973) observed a concentration factor of 1.7 when he fed dieldrin-contaminated brine shrimp, *Artemia salina*, nauplii (0.213 $\mu g/g$) to crab larvae. Macek and Korn (1970) observed a food concentration factor of 0.6 for brook trout exposed to 3 $\mu g/g$ DDT in their diet for 120 days. Grzenda et al. (1970) estimated a total mean body residue level of 14.2 $\mu g/g$ DDT in goldfish after they were fed a diet containing 17.7 $\mu g/g$ DDT for 192 days, which would indicate a concentration factor of 0.8.

Some ^{14}C-DDT that leached from the food into the water was observed by liquid scintillation, and a mean ^{14}C-DDT water concentration of 0.065 ± 0.007 $\mu g/\ell$ ($n = 12$) was determined. If this level was bioaccumulated 100,000 times, the highest level that could occur in the fish would be 6.5 $\mu g/g$, or about 10% of the total food-contributed residue. However, the total DDT concentration measured in the water by gas-chromatographic analysis was not much greater when the fish were exposed to DDT-contaminated food only. If the control fish with a mean measured water concentration of 0.0029 $\mu g/\ell$ DDT are compared with fish exposed only to DDT in the food (F) with a mean measured DDT water concentration of 0.0123 $\mu g/\ell$, the difference is only 0.009 $\mu g/\ell$, a possible body residue of 0.9 $\mu g/g$ or about 1.5% of residues caused by DDT in the water leached from the food (assuming a 100,000 times magnification). Therefore, we believe that the contribution of labeled DDT through the water was negligible.

Fish exposed to DDT in the water exhibited higher total DDT residues at 7 days than at 14 days. This abnormal uptake pattern was caused by a decrease in lipid content of the fish which resulted from decreased food consumption. When the fish were first fed clam meat they would not feed; subsequently, a decrease of percent lipids resulted. When the fish began to feed normally, their lipid content stabilized and DDT tissue residues increased. Percent lipids changed similarly for the F exposed fish but since the only source of DDT exposure was the food, these fish showed a more typical DDT uptake.

Our results indicate that DDT in the tissues decreased between 14 and 266 days. During the same period DDE and TDE residues increased. Grzenda et al. (1970) observed similar results for goldfish fed a DDT-contaminated diet for up to 192 days. We also observed in our test that the TDE residues were high in fish exposed to dietary DDT and were low in fish fed clean food. This indicates that most of the TDE in the fish fed a contaminated diet probably came from the food itself, which was quite high in TDE.

The clams metabolized DDT almost entirely to TDE and produced little DDE. Some discrepancy was observed in the amounts of TDE produced between exposures. Although all clam DDT exposures were held at the same temperature (20°C), clams for exposures 1 and 4 were collected in the fall and exposed in the spring, whereas clams for exposures 2 and 3 were collected in the spring and fall and exposed in the fall and winter. More clam metabolism would naturally occur in the spring than in the winter, and a seasonally controlled mechanism might be responsible for metabolite differences among the DDT-exposed clams. Another possible explanation is that differ-

ences could have been caused by different ratios of clam species present. No check was made as to the relative frequency of each species, although the genus *Lampsilis* appeared to be most prevalent. The 1 μg/g DDT found in the control fish was probably caused by the presence of 0.7 μg/g DDT in the first batch of clean clam tissue fed to the fish. This explanation is likely since the use of this clam food and the residue peak in the control fish terminated at about the same time.

The proportion of TDE and DDE produced appears to vary with the type of organism exposed. Rats convert most DDT to TDE, whereas humans usually metabolize DDT to DDE (O'Brien 1967). Molluscs, as observed in this test and by Cooke and Pollard (1973), metabolize DDT mainly to TDE, whereas fish metabolize DDT essentially to DDE (Priester 1965; Johnson and Pecor 1969; Reinert and Bergman 1974). Ferguson et al. (1967), however, observed almost equal amounts of DDT, DDE, and TDE in several pooled samples of resistant mosquitofish (*Gambusia affinis*). Therefore, some fish can also readily produce large amounts of TDE.

Some breakdown of DDT to TDE may have occurred in our test when the clam tissue was oven-dried or when it was in frozen storage. Breakdown of DDT to TDE in frozen storage was demonstrated by French and Jefferies (1971). Our clam tissue, however, was used within 60 days, so postmortem breakdown is believed to have been negligible. Oven-drying also is not believed to have caused much DDT breakdown. Smith et al. (1973) found no large change in total TDE after fish steaks were baked at 177°C, and Metcalf (1955) states that pure DDT is stable up to 195°C.

Lipid values could be correlated with residue values, an indication that DDT uptake was influenced by the lipid content of the fish. DDT-residue levels declined rapidly during the spawning period. Lipid content of the fish also decreased at this time in fish exposed to the 2.0 μg/ℓ DDT water concentration whether they were fed clean or DDT-contaminated food. This is probably a fairly common occurrence. Reinert and Bergman (1974) observed that DDT residues were redistributed in the tissues of spawning-run fish and that this redistribution was closely related to a general decrease in the amount of fish oil.

The presence of DDT in the diet significantly reduced the probability of survival for exposed fish. Two definite periods of death were observed, the early larval stage up to 73 days of age and the spawning period, when mature males were the most sensitive. Fish that died during spawning were in relatively poor condition and did not feed. They probably used their fat reserves, thereby causing a release of stored DDT into the blood where the DDT could become toxic. Holden (1962) stated that fish in poor conditon or with low fat content were more susceptible to DDT toxicity. Our results support Holden's statement in that fish that died had predominantly lower lipid values than live fish sampled at the same time. Redistribution of DDT to the brain during weight loss with resultant deaths has been observed by some authors. Dale et al. (1962) observed high brain levels of DDT in rats that were starved after being fed DDT. Transfer of DDT to brains of birds and some resultant deaths were observed by Bernard (1966), Ecobichon and Saschenbrecker (1969), and Van Velzen et al. (1972). Redistribution of DDT and fat depletion in salmon and trout were observed during spawning run by Holden (1962) and Reinert and Bergman (1974) and in the laboratory in starved rainbow trout by Grant and Schoettger (1972). Desaiah et al. (1975) analyzed fish sampled at 56, 118, 225, and 266 days during our toxicity test and found that partial chronic exposure to DDT significantly inhibited mitochondrial Mg^{2+}-ATPase activity in the brain tissue of the fathead minnows.

In regard to toxicity in relation to the high concentrations of TDE in the food, the oral toxicity of TDE to rats (U.S. EPA 1972) is about one third that of DDE. In bluegills, however, the static acute data of Cope (1965) and Foster Mayer (Fish-Pesticide Research Laboratory, Columbia, Missouri 65201, personal communication) indicate that TDE is more toxic than DDE. Probably the relative toxicity of each varies with the specific organism exposed. Both DDE and TDE, however, appear to be less toxic than DDT (Oettingen and Sharpless 1946; Rudd and Genelly 1956; O'Brien 1967; Moyle and Skrypek 1969). Therefore, even though our clam tissue had high levels of TDE, our mortality results present a more accurate example of the DDT effect in a normal aquatic food chain than if we had used the more toxic unmetabolized DDT in a dry food mix.

To estimate the relative contribution of total DDT from the food to DDT from the water in regard to its effect on mortality the following model was assumed: the log of the total dose that a fish can tolerate has a normal distribution which can be approximated closely by a logistic distribution (Berkson 1951, 1953, 1955; Finney 1964). Thus, we have that

$$(1) \quad \ln(P/1 - P) = \gamma + \beta \ln x$$

where x is the total dose delivered to the fish and

P is the probability of death given a dose at x. It was further assumed that DDT in water and food acts on the fish in a similar manner and thus x is proportional to the sum of water and food exposure and is expressed as

$$(2) \quad x = \theta\,(x_1 + \rho x_2)$$

where θ is a magnification and absorption factor in the target organism, x_1 = concentration of DDT in the water, $x_2 = 1$ if DDT is in the food, zero if otherwise, and ρ is the unknown amount of DDT ingested from the food. Substituting the value for x into equation (1) we have that

$$(3) \quad \ln(P/1-P) = \gamma + \beta\ln(\theta(x_1 + \rho x_2)) = \alpha + \beta\ln(x_1 + \rho x_2)$$

where $\alpha = \gamma + \beta\ln\theta$ is a combination of parameters that are estimable from the data. The probability of death, given a target dose of x, may be estimated from our data utilizing Abbott's formula (Finney 1971) from the relationship $Ps = (1 - Po)\,(1 - P)$, where Po is the probability of death of a control fish. Thus $P = 1 - Ps/(1 - Po)$ is an estimated probability of death due to DDT. Table 9 shows these estimated P values, the actual mean measured DDT water concentrations in duplicated exposure chambers, and the values of the estimated parameters obtained using nonlinear least square estimation. We used nonlinear estimation because ρ enters into the equation in a nonlinear manner, i.e. the amount of DDT ingested in the food and its effect on toxicity is unknown as compared to DDT sorbed from the water and this had to be estimated in the equation. The value $e^{-\alpha/\beta}$ is an estimate of LC50 caused by total exposure (Berkson 1944, 1953). It is estimated as 1.4596 $\mu g/\ell$ for DDT in water with no DDT in the food, or 0.9270 $\mu g/\ell$ for DDT in water with 45.6 $\mu g/g$ DDT in the food. The amount of DDT in the food delivered to the target organism in this study is estimated as 0.5325 U in water. Thus, the percentage of DDT in the fish from DDT in the contaminated food in the 0.5 and 2.0 $\mu g/\ell$ DDT water exposures is estimated as 58.8 and 26.4, respectively. These estimates are very close to the measured percentages determined by liquid-scintillation analyses as shown in Table 4. The estimated relationship between probability of death and DDT in the water with and without DDT in the food indicates that at low DDT water concentrations the importance of the DDT-contaminated food is greater, and as the DDT water concentration increases the importance of the food effect decreases (Fig. 4). Dunnett's procedure (Steel and Torrie 1960) was used to compare the mean probability of the combined duplicate test exposure survival with that of the con-

TABLE 9. Estimated P values, average measured DDT water concentrations, and values for estimated parameters from nonlinear least square estimation for fish exposed to various test conditions.

Estimated P of death caused by DDT	$\ln(P/1-P)$	X_1 Avg measured DDT in the water ($\mu g/\ell$)	X_2 (0 if no DDT in food) (1 if DDT in food)
0.0598	−2.7550	0.3517	0
0.4680	−0.1280	1.5285	0
0.1489	−1.7430	0.0123	1
0.1685	−1.5960	0.3740	1
0.7534	1.1170	1.4839	1

$$\ln(P/1-P) = \alpha + \beta\ln(X_1 + \rho X_2)$$

Parameters	Estimates of parameters	(SD)
α	−0.7661	(0.3802)
β	2.0261	(0.4313)
ρ	0.5325	(0.1946)

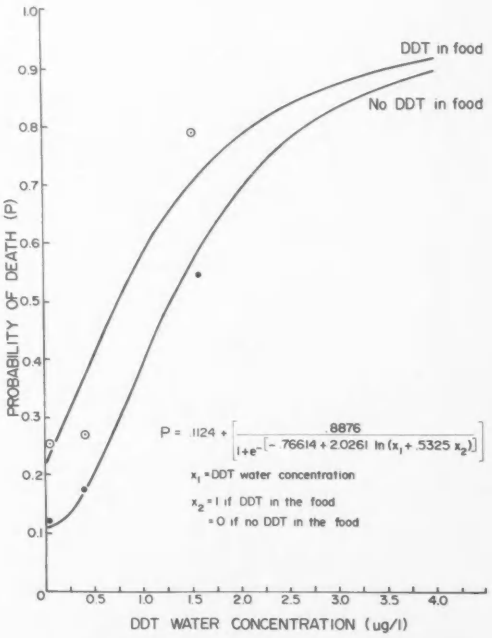
DDT in food
No DDT in food
PROBABILITY OF DEATH (P)
DDT WATER CONCENTRATION (ug/l)
P = .1124 + .8876 / (1+e^-[-.76614 + 2.0261 ln(x1 + .5325 x2)])
x1 = DDT water concentration
x2 = 1 if DDT in the food
= 0 if no DDT in the food

FIG. 4. Estimated probability of death for fish exposed to DDT at various water concentrations and fed clean or DDT-contaminated food.

trols. With $P = 0.05$, reduction in the probability of survival was significant only at the 2.0 W and 2.0 W + F exposures. By back calculation it is estimated that reduction in the probability of survival to 64.5% (35.5% mortality) is necessary to be significantly different. This mortality level would fall at about 0.425 $\mu g/\ell$ DDT in the water plus DDT in the food and at 0.925 $\mu g/\ell$ DDT in the water without DDT in the food (Fig. 4).

Our mortality data indicate that the presence of dietary DDT is more important when DDT water concentrations are low. A similar situation in nature could easily occur if food organisms built up tissue residues during pesticide contamination and then were eaten by predators that entered the area after pesticide water concentrations had moderated, or if several successive food-chain accumulations occurred. Dietary DDT exposure is important at relatively higher water concentrations, although to a lesser degree than water exposure. This view is also held by other investigators, such as Grzenda et al. (1970), who stated that apparent DDT biological magnification by the food chain may not be as significant as the length of time fish are exposed to pesticide residues in the water; Reinert and Bergman (1974), who stated that rapid accumulation of DDT residues in coho salmon was probably related in part to an increase in the intake of DDT-contaminated alewives; and Macek and Korn (1970), who demonstrated that dietary DDT can be very important, especially when compared with very low DDT water concentrations such as are found in Lake Michigan.

Embryo hatchability was reduced significantly only when parent fish were exposed to DDT at 2.0 $\mu g/\ell$ in the water and fed clean food. However, since DDT tissue residues were twice as high in embryos from fish exposed to DDT both in the diet and at 2.0 $\mu g/\ell$ in the water, the significant reduction of hatchability in the former group and not in the latter may have been the result of adult fathead minnow variability. Residues caused by dietary DDT were additive for all embryos from parent fish exposed to DDT in water and food. Huisman et al. (1971) reported that high residues of DDT, DDE, and TDE were accompanied by low fertility in pike. Kleinert and Degurse (1973) demonstrated a strong correlation between DDT content of embryos and larvae from walleyes in Wisconsin lakes, but could not associate the presence of DDT with success of the hatch. Burdick et al. (1972) demonstrated loss of brown trout and brook trout larvae hatched from eggs taken from female fish fed dietary DDT at different concentrations and durations of time. They also confirmed DDT as the cause of 100% mortality of larvae reared from eggs from lake trout fed 6 μg DDT/g body wt per wk. The average DDT residues in eggs less DDE were 7.61 and 11.92 $\mu g/g$ for 2 separate yr. In our study mean total DDT residues in embryos less DDE were 4.31 $\mu g/g$ from fish fed dietary DDT and 9.55 $\mu g/g$ from fish fed dietary DDT and also exposed to DDT in the water. Lack of 100% larval mortality at these levels is probably explained by species variability, as fathead minnows are generally less susceptible to DDT than salmonids. Larvae from fish exposed at 2.0 W and 2.0 W + F reared at the same water concentration as the parent fish experienced 100% mortality within 5 days. Part of this mortality may have been caused by DDT sorbed from the water, as larvae from these two parental groups transferred to control water for 30 days demonstrated an average of only 59% mortality among those from parents exposed to DDT in the water only and $> 81\%$ mortality among those from parent fish exposed to DDT in both the water and diet, an almost twofold higher mortality. These mortality data agree closely with embryo residue results that indicated almost twice as much DDT residue (40.9 vs. 24.0 $\mu g/g$) in embryos from parent fish exposed to DDT in both water and diet. Although residue levels were high, the larvae could either readily eliminate the DDT or dilute the residues through growth if placed in clean water. After 30 days in clean water these larvae, if they survived, had residues no greater than those in control larvae.

Total DDT residues were higher at all life stages whenever parent fish were exposed to DDT in both the water and diet than for water exposure alone. Even though the concentrations of DDT accumulated from water are higher than those accumulated from food, the presence of DDT in the diet caused higher tissue residues and death rates than were found in fish that were only exposed to DDT in the water. Death rates were not significant for larvae exposed to dietary DDT at 30 and 60 days, perhaps because some larvae could not adapt to clam tissue when active feeding commenced, and deaths occurred among the control fish by starvation. This was reflected in a mean mortality of 34% among control larvae at 30 days.

A "just safe" water concentration or maximum acceptable toxicant concentration (MATC) determined from mortality results would be about 0.9 $\mu g/\ell$ DDT for water exposure alone and about 0.4 $\mu g/\ell$ DDT with the added presence of dietary DDT. Application factors as defined by Mount and Stephan (1967), using a 96-h TL50 value of 48 $\mu g/\ell$ DDT demonstrated in one of

our acute fathead minnow toxicity tests and the maximum acceptable toxicant concentrations above, would be 0.0188 or 1/53 for fish exposed to DDT in the water alone and 0.0083 or 1/120 for fish exposed to DDT in both the water and diet. Consequently, food as well as water sources of exposure to certain materials must be considered when toxicity tests are designed or the conclusions drawn from such tests are evaluated.

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Blood Chemistry, Biochemical Composition, and the Annual Reproductive Cycle in the Giant Scallop, *Placopecten magellanicus*, from Southeast Newfoundland[1]

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THOMPSON, R. J. 1977. Blood chemistry, biochemical composition, and the annual reproductive cycle in the giant scallop, *Placopecten magellanicus*, from southeast Newfoundland. J. Fish. Res. Board Can. 34: 2104–2116.

Gametogenesis in the giant scallop, *Placopecten magellanicus* (Gmelin), occurs in early summer and spawning takes place during August and September. The gonad differentiates between March and May, when the water temperature is low (0 to −1°C) and food supply high. Gonad growth also begins at this time. Energy reserves from the previous year do not seem to play a significant part in subsequent growth and maturation of the gonad. There is no accumulation of metabolic reserves in the scallop gonad after spawning; in winter, the gonad is very small and inactive. Levels of lipid (mostly phospholipids and free sterols), protein, low molecular weight carbohydrate, and glycogen increase in the plasma during the period of gonad growth and maturation. The amount of glycogen in the circulating hemocytes is greater in summer than in winter.

Key words: *Placopecten magellanicus*, bivalve, scallop, reproductive cycle, blood, hemolymph, hemocyte, blood chemistry, glycogen, phospholipid, sterol

THOMPSON, R. J. 1977. Blood chemistry, biochemical composition, and the annual reproductive cycle in the giant scallop, *Placopecten magellanicus*, from southeast Newfoundland. J. Fish. Res. Board Can. 34: 2104–2116.

La gamétogénèse chez le pétoncle géant, *Placopecten magellanicus* (Gmelin), se produit au début de l'été, et la ponte a lieu en août et septembre. La gonade se différencie entre mars et mai, au moment où la température de l'eau est basse (0 à −1°C) et les approvisionnements en nourriture sont élevés. La croissance de la gonade commence également à cette époque. Les réserves énergétiques de l'année précédente ne semblent pas jouer un rôle important dans la croissance subséquente et la maturation de la gonade. Il n'y a pas accumulation de réserves métaboliques dans la gonade du pétoncle après la ponte; en hiver, la gonade est très petite et inactive. Les niveaux de lipides (en grande partie phospholipides et stérols libres), de protéines, d'hydrates de carbone de faibles poids moléculaires et de glycogène augmentent dans le plasma durant la période de croissance et de maturation de la gonade. La quantité de glycogène dans les hémocytes en circulation est plus grande en été qu'en hiver.

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THE importance of stored energy reserves in the annual reproductive cycles of marine bivalves from temperate regions is now well documented, particularly for the blue mussel, *Mytilus edulis* (Bayne and Thompson 1970; Gabbott and Bayne 1973; Gabbott 1975, 1976). In the mussel, reserves are accumulated during the summer and autumn and are utilized in the winter, when food is less plentiful and gametogenesis is taking place. Similar but less comprehensive observations have been made on other bivalves. In the Pectinidae, seasonal changes in biochemical composition have been described for *Chlamys septemradiata* by Ansell (1974) and for *Pecten maximus* by Comely (1974). The roles of environmental factors in the regulation of gonad development in scallops have been reviewed by Sastry (1975).

This paper describes the seasonal changes in carbohydrate, lipid, and protein levels in the gonad and somatic tissue of the giant scallop, *Placopecten magellanicus*, from southeast Newfoundland. Seasonal changes in blood chemistry are also described, and discussed in terms of the annual reproductive cycle.

Materials and Methods

COLLECTION OF ANIMALS

Scallops were collected periodically (by SCUBA) from three localities in southeast Newfoundland: North Harbour (Placentia Bay), Dildo (Trinity Bay), and Salmonier (St. Mary's Bay). Only large

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scallops were taken — 14–18 cm long (i.e. from anterior margin to posterior) at North Harbour and Salmonier; 16–19 cm long at Dildo. The animals were transported to the laboratory in seawater and then placed in tanks at ambient temperature.

Removal of Tissues

The soft tissues were removed from three males and three females in each collection and divided into two components, gonad and somatic tissue. Six gonad samples and six somatic tissue samples were thus obtained from each group of scallops acquired. The material was dried to constant weight at 90°C.

Chemical Analysis of Tissues

Dried gonad and somatic tissue from North Harbour scallops was homogenized in a ball mill before chemical analyses were carried out. Carbohydrate, lipid, protein, and ash were determined as described by Thompson et al. (1974). DNA was extracted from the male gonad by a procedure modified from Munro and Fleck (1966). Approximately 0.1–0.2 g wet tissue was excised from the freshly killed animal and placed on ice. The tissue was broken up in 2 ml ice-cold water by means of ultrasonic disintegration. Temperature was maintained between 0 and 4°C. Protein and nucleic acids were then precipitated by the addition of 5 ml 13% trichloroacetic acid (TCA). The samples were kept in ice for 10 min, then centrifuged. The precipitate was washed with 10% TCA before incubation in 2 ml 1.2 M perchloric acid (20 min at 70°C). Insoluble material was then removed by centrifuging the sample. DNA was determined in the supernatant by the diphenylamine method (Giles and Myers 1965), using calf thymus DNA (Sigma Chemical Co., St. Louis, Mo.) as the standard.

Removal of Blood

Blood samples (12 ml each) were taken from the adductor sinuses of three male and three female scallops (North Harbour population) within 24 h of collection. The samples were centrifuged (10 min, 2000 *g*) to separate hemocytes from plasma. A portion of the plasma (usually 3 ml) was then deproteinized by the addition of an equal volume of 20% TCA and kept for several hours at 4°C. This fraction, after removal of the protein by centrifuging, was used for the determination of plasma nonprotein nitrogen, nonprotein Lowry positive substances, and carbohydrate. The untreated plasma fraction was used immediately for estimation of total Lowry positive compounds, ammonia nitrogen, and lipid. Tubes containing the hemocyte pellets were inverted for 5 min to drain them of residual plasma. The hemocytes were weighed and stored at −22°C for chemical analysis, which was always carried out < 48 h after the blood sample was taken. Since the volume of cells per unit volume of blood was too small to measure in a standard hematocrit tube, hematocrit was calculated as the hemocyte pellet weight expressed as a percentage of the weight of the blood sample.

Plasma Analyses

Lipids were extracted from 0.5 ml plasma with 1:2 vol/vol chloroform: methanol and quantified by charring at 180°C with concentrated sulphuric acid, using tripalmitin as a standard (Holland and Gabbott 1971). To confirm the effectiveness of this procedure, lipids were extracted from 100 ml plasma and determined gravimetrically. A replicate sample (0.5 ml) was also taken through the extraction, but lipid was estimated by charring with sulphuric acid. A value of 9.6 mg lipid · 100 ml^{-1} was obtained from the latter procedure, compared with 9.1 mg · 100 ml^{-1} for the gravimetric determination.

Ammonia nitrogen was determined by the phenol–hypochlorite reaction (Grasshoff and Johannsen 1972). Glycogen was precipitated from deproteinized plasma by the addition of ethanol (final concentration 70%) and removed by centrifuging (20 min, 3000 *g*). This procedure recovered 94% of the oyster glycogen (BDH Ltd., Poole, U.K.) dissolved in 10% TCA (gravimetric determination). The glycogen precipitate was washed with 70% ethanol and dissolved in 5% TCA. Low molecular weight carbohydrate (in the ethanol supernatant) and glycogen were estimated separately by the phenol–sulphuric acid procedure (Dubois et al. 1956). For the determination of nonprotein nitrogen, 2 ml deproteinized plasma were digested in 2 ml concentrated sulphuric acid containing 0.02% wt/vol selenium dioxide as a catalyst; the digest was neutralized and the ammonia nitrogen content estimated by the phenol–hypochlorite reaction. Lowry determinations were carried out on untreated plasma and deproteinized plasma; protein was estimated by difference.

Hemocyte Analyses

Cell pellets were sonicated briefly (5 s) in 1 ml water. Lipids were extracted from an aliquot of the homogenate and quantified using procedures described for plasma. Protein was precipitated from the remainder of the homogenate by the addition of an equal volume of 20% TCA at 2°C. The centrifuged protein precipitate was washed with 10% TCA, hydrolyzed in 0.5 M potassium hydroxide and protein determined by the Lowry method. Glycogen and low molecular weight carbohydrate (in the supernatant) were estimated separately as described for plasma.

Effects of Collection Procedure on Blood Chemistry

Blood samples were removed from 10 scallops within 30 min of collection. Hemocytes were immediately separated from the plasma, and the cell pellets placed in dry ice. Determinations of ammonia nitrogen and Lowry positive substances in the plasma were made at the collection site. Deproteinization of the plasma and extraction of plasma lipid were also carried out in the field. The scallops were marked and transported to the laboratory, where analyses of the plasma and frozen hemocytes were completed as previously described. Twenty-four hours after collec-

tion of the scallops, blood samples were taken from the marked animals (now held in the laboratory) and the entire analytical procedure repeated. Mean values for metabolite concentrations in field and laboratory groups were then compared using the *t*-test appropriate for a "self-pairing" design.

Chromatography of Lipids

Lipid extracts from plasma and hemocytes were stored at −20°C in chloroform containing 0.001% wt/vol butyl hydroxytoluene as an antioxidant. Neutral lipids were separated on 20 × 20 cm thin-layer plates using a two-step, one-dimensional procedure described by Skipski et al. (1965). Plates were washed before use by development in 3:1 chloroform:methanol, followed by removal of a 1-cm strip of silica gel from the top of the plate. Lipid samples were dried in a vacuum oven at 40°C to remove the antioxidant, taken up in 20 μl chloroform and applied to activated, cooled plates. After separation of the lipids (Skipski et al. 1965), dried plates were exposed to ultraviolet light and fluorescent spots marked with a needle. Silica gel from each spot was drawn onto a sintered glass disc by suction and the lipids eluted with dichloromethane:methanol (9:1). Eluates were evaporated to dryness in a stream of nitrogen and immediately charred with sulphuric acid (as described above) for quantitative analysis of the neutral lipid classes. Blanks were obtained by elution and charring of nonfluorescent regions of each plate. Lipid standards (monopalmitin, dipalmitin, tripalmitin, palmitic acid, palmitic acid methyl ester, cholesterol, and cholesterol palmitate) were obtained from the Sigma Chemical Co. All solvents used for lipid extraction, chromatography, and elution were distilled before use.

Phospholipids were separated by thin-layer chromatography in a two-dimensional system described by Abdel-Latif and Chang (1966). Neutral lipids were removed by development in acetone:petroleum ether 1:3 before separation of phospholipids in chloroform:methanol:acetic acid:water 25:15:4:2 (first dimension) and butanol:pyridine:water 45:5:20 (second dimension). Plates were then dried and sprayed with a molybdate reagent to detect phospholipids. A second solvent system (chloroform:methanol:ammonium hydroxide:water 120:60:5:5) was also used to separate phospholipids (Wheeler and Whittam 1970). No attempt was made to distinguish between phosphatidyl and phosphatidal (plasmalogen) forms.

Counting and Sizing of Hemocytes

One-half millilitre of blood was drawn into a syringe containing 0.5 ml 0.5% *n*-ethyl-maleimide in seawater. The syringe was immediately inverted several times and vigorously agitated to ensure complete mixing of the sample. Inspection by microscopy confirmed that negligible aggregation of hemocytes occurred when this procedure was followed. The contents of the syringe were then added to 50 or 60 ml 0.2% *n*-ethyl-maleimide in seawater, and the suspension mixed thoroughly. Hemocytes were counted with a Coulter counter model Z_B. No decrease in cell numbers was noted in successive counts of any given sample, further confirming the effectiveness of the anticlumping agent. Size-frequency distributions of the hemocytes in each sample were obtained with a Coulter counter channelyzer calibrated with mulberry pollen. All *n*-ethyl-maleimide solutions were made up in membrane filtered (0.45 μm) seawater and kept at 4°C.

Results

Reproductive Cycle

Placopecten magellanicus exhibits a distinct annual reproductive cycle. Gonad growth and maturation take place between April and July (Fig. 1). Spawning occurs in August and September and is followed by a long period during which gonad dry weight is very low. The weight of the somatic tissue is also low during the winter, increasing during April and May. Seasonal changes in the gonad weight index are similar in the three populations examined (Fig. 2). The summer maxima in gonad indices were lower in 1975 than in 1974.

Measurements of DNA in the male gonad (Fig. 3) indicate that differentiation begins in February or March and accelerates in April. Gonad maturation is complete in July. These observations confirm those of Naidu (1970), which were based on histological examination of developing gonads in *P. magellanicus* from western Newfoundland.

Biochemical Composition of Tissues

There are some difficulties in the presentation of tissue composition data (Gabbott 1976). Carbohydrate, lipid, and protein content are often reported as percentages of tissue weight, but this may lead to problems in interpretation, since changes in the concentration of any given constituent result in reciprocal changes in percent by weight values for all the others. Alternatively, data may be presented as the total weight of a constituent present in a tissue or animal, but this approach is complicated by seasonal growth patterns or weight losses and often does not facilitate comparisons between studies, particularly of different species. Both forms of data presentation have been employed in this work on *P. magellanicus* and have served to complement one another.

The level of carbohydrate in the gonad is low, varying from 3% (winter) to 5% (summer) of the tissue dry weight (Fig. 4). In winter the gonad contains very little carbohydrate, but the

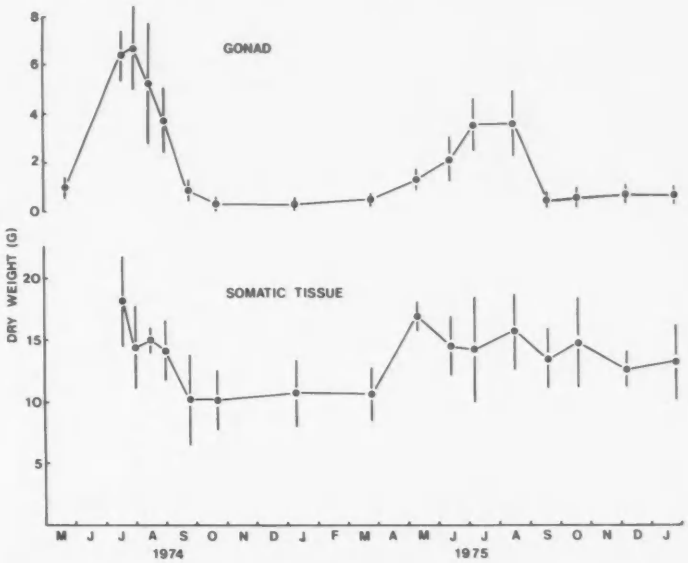

FIG. 1. Gonad and somatic tissue dry weights for North Harbour scallops. Means ± 95% confidence limits.

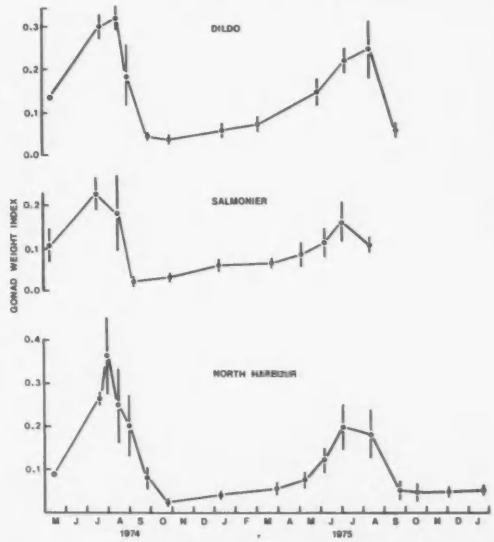

FIG. 2. Gonad weight index (dry weight of gonad divided by dry weight of soft tissues) for three populations of *Placopecten magellanicus*. Means ± 95% confidence limits.

carbohydrate content increases as the gonad develops, reaching a maximum in July and August (Table 1).

In the female gonad the lipid level is lowest in the winter, increases during gonad maturation, and reaches a maximum (approximately 20% of tissue dry weight) in July. In 1974 the amount of lipid in the female gonad, relative to other constituents, remained high after spawning was complete, whereas in the following year lipid decreased from 17 to 7% of dry weight during the spawning period (Fig. 4). In both years, however, the bulk of the lipid accumulated during development of the female gonad was released in the spawned eggs (Table 1). There is less seasonal variation in the proportion of lipid in the

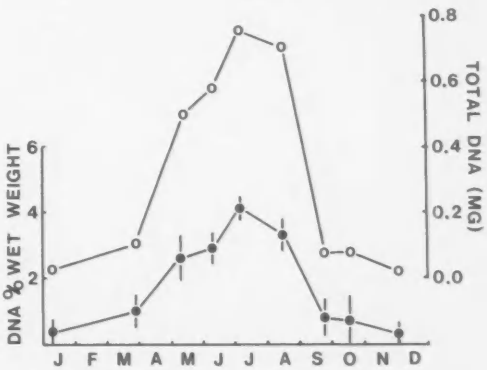

FIG. 3. DNA in the male gonad, expressed as a percentage of tissue wet weight (●; means ± 95% confidence limits) and as the total present in the tissue (○).

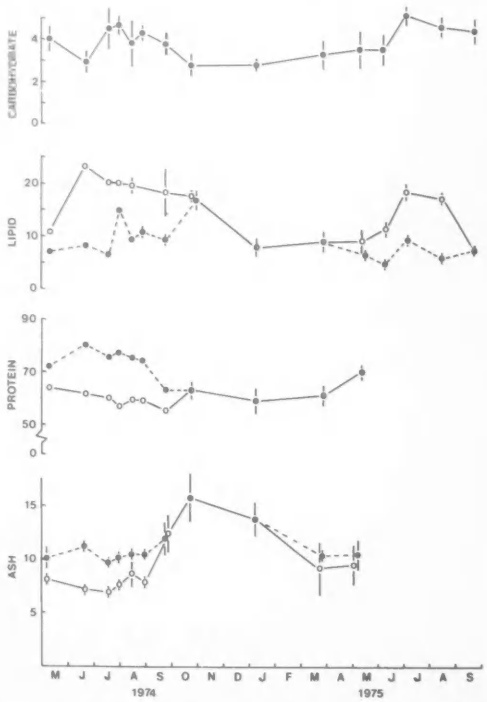

FIG. 4. Biochemical composition of the gonad. Components are expressed as percentages of tissue dry weight (means ± 95% confidence limits). Females –○–, males – – –●– – –, except for carbohydrate data (values for males and females pooled).

male gonad, although the lipid level was high in October 1974, immediately after completion of spawning (Fig. 4).

Protein levels in the gonad are characterized by higher values in the ripe male than in the female at the same stage (Fig. 4). This presumably reflects the high nucleoprotein content of the male gametes, whereas the eggs contain lipid yolk reserves and relatively less protein than the sperm.

Ash content of the gonad is greatest immediately after spawning, decreases gradually as the winter progresses, and reaches minimum values during gonad maturation. Throughout the summer the male gonad contains less inorganic matter than the female gonad.

Carbohydrate represents a greater proportion by weight in the somatic tissue than in the gonad (Fig. 5). The concentration of carbohydrate is greatest in June, decreasing during the spawning period and throughout the winter to a minimum in March. The level of carbohydrate rises rapidly during early summer. The carbohydrate content of the somatic tissue is considerably lower in the winter than in the summer (Table 1).

Lipid makes up 8–9% of the somatic tissue dry weight throughout the year, although there was a decrease after spawning in 1975 (Fig. 5). However, low values for somatic tissue weight in winter result in reduced lipid content at this time.

Protein in the somatic tissue represents 58–64% of total dry weight; it is slightly lower in the autumn and winter than in the summer.

The proportion of ash in the somatic tissue is greatest immediately after spawning takes place, decreasing during winter and reaching minimum values in early summer. This seasonal pattern is similar to that observed in the gonad.

BLOOD CHEMISTRY

Coefficients of variation (C.V. = SD/$\bar{x}$) were determined for each assay, using 10 replicates of a single plasma sample in each case:

	C.V.
Lowry	6.3%
Carbohydrate	6.7%
Ammonia nitrogen	16.9%
Lipid (including extraction)	8.5%
Nonprotein nitrogen (including digestion)	5.7%

To establish a satisfactory procedure for collecting scallops and taking blood samples, blood was removed on one occasion from animals immediately after collection and from the same individuals 24 h later in the laboratory. Lipid levels in the plasma were significantly lower in the laboratory samples than in those from the field, but there was no significant difference between means for the other metabolites (Table 2). Bayne (1973) has demonstrated a reduction in plasma lipid during starvation of *M. edulis* in the laboratory. The small decrease observed in *P. magellanicus* plasma lipid after 24 h in the laboratory may reflect the beginning of a similar trend. Alternatively, lipid levels in the plasma may have been temporarily elevated due to disturbance of the scallops during collection.

A single classification analysis of variance carried out for each metabolite, with values grouped by sampling date, showed a significant seasonal effect on all plasma metabolites and on hemocyte low molecular weight carbohydrate, glycogen, and total carbohydrate (Table 3). Similar results were obtained by Bayne (1973) for plasma metabolites of *M. edulis*.

Ammonia nitrogen in the plasma varied between 0.4 and 0.9 mg·100 ml^{-1} throughout the winter, increased to 1.6 mg·100 ml^{-1} in early June, fell to a minimum value of 0.2 mg·100

TABLE 1. Carbohydrate, lipid, and protein content of scallop tissue (milligrams per animal). Values are means ± SD.

Date	Sex	Gonad			Somatic tissue		
		Carbohydrate	Lipid	Protein	Carbohydrate	Lipid	Protein
May 7, 1974	♀	59± 8	145± 37	856± 112	1766± 668	1260±426	9152± 891
	♂		106± 19	1087± 85			
June 20, 1974	♀	57± 11	391± 89	1013± 110	1538± 268	1180±325	7563±1391
	♂		212± 19	2018± 29			
July 18, 1974	♀	280± 32	1416±438	4140± 773	2148± 591	1534±476	11414±2690
	♂		407± 45	4431± 493			
July 30, 1974	♀	305± 89	1228± 2	3045± 817	1782± 849	1265±602	9207±1989
	♂		1187± 83	6115± 504			
Aug. 15, 1974	♀	415±296	1640± 14	3588±1961	3090±1091	1471±351	9265±1094
	♂		426±196	3898±1138			
Aug. 28, 1974	♀	164± 79	1281±269	2681± 480	1285± 529	1200±252	9289±1728
	♂		288±129	1970± 949			
Sept. 23, 1974	♀	33± 17	168±136	507± 214	968± 497	1541±817	6090±2307
	♂		89± 27				
Oct. 25, 1974	♀+♂	7± 3	41± 12	150± 49	865± 272	817±221	6327±1581
Jan. 9, 1975	♀+♂	12± 2	33± 11	249± 43	858± 301	920±386	7033±1693
Mar. 27, 1975	♀+♂	19± 9	51± 24	341± 98	537± 360	952±328	6151±1225
May 8, 1975	♀+♂	44± 13	102± 36	841± 237	1767± 86	1463±165	9314± 365
June 5, 1975	♀	69± 33	178± 63		1953± 295	1107±166	
	♂		105± 38				
July 4, 1975	♀	169± 58	481±193		1816± 604	1274±516	
	♂		394±127				
Aug. 14, 1975	♀	150± 70	460±303		1073± 230	1420±403	
	♂		249± 56				
Sept. 23, 1975	♀+♂	12± 6	29± 16		751± 370	633±239	

ml^{-1} in August and then increased to reach a maximum of 2.1 mg·100 ml^{-1} in November (Fig. 6). These values are similar to those recorded by Bayne (1973) for *M. edulis*, in which ammonia nitrogen is low in the summer and high in the autumn.

Plasma protein increased from 90 mg·100 ml^{-1} in February to 250 mg·100 ml^{-1} in June, decreased in July, and then rose to high values again in the autumn (Fig. 6). Nonprotein Lowry positive substances (mostly polypeptides, peptides and free tyrosine) made up 36% of the total Lowry fraction in February, decreasing to 2% in June. There was a further increase in nonprotein Lowry positive compounds during July and August, followed by a reduction in September. Bayne (1973) found total Lowry substances in *M. edulis* plasma to be low in midsummer and high in autumn, which corresponds with the cycle in *P. magellanicus*.

Nonprotein nitrogen in the plasma was a maximum in February, decreasing steadily to minimum values in May, and then rising slightly to a level maintained throughout autumn.

Plasma lipid was lowest in March, but increased considerably during early June. Lipid levels in the plasma remained high throughout summer, but decreased after the spawning period in autumn; there was a further reduction in plasma lipid during the late winter. In the mussel, Bayne (1973) obtained higher values for plasma lipid (20 mg·100 ml^{-1} in summer to 84 mg·100 ml^{-1} in autumn) than those recorded here for the scallop, but this may be attributable to differences in methodology.

In the plasma, low molecular weight carbohydrate (i.e. carbohydrate remaining in solution after precipitation of glycogen in 70% ethanol) was highest in late summer, decreasing through the autumn and winter to reach lowest levels in April and May. Plasma glycogen remained low (<2 mg·100 ml^{-1}) during late summer, autumn, and winter, but increased in April and May to a peak of 8.5 mg·100 ml^{-1} at the beginning of June. Low molecular weight compounds accounted for 70–90% of the carbohydrate in the plasma for most of the year, but constituted only 40% of the total in May, when the glycogen levels were high. According to Bayne (1973) blood sugar in *M. edulis* (determined with the aniline reagent) varies from 10 to 36 mg·100 ml^{-1}, with high values occurring in late spring and autumn, and low values in midsummer and midwinter.

There were no seasonal changes in hematocrit or in the lipid and protein levels in the hemocytes (Fig. 7, Table 3). Levels of low molecular weight

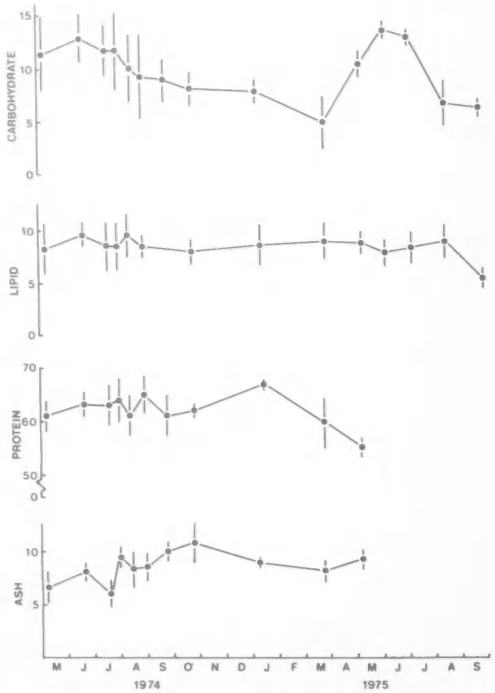

FIG. 5. Biochemical composition of somatic tissue. Components are expressed as percentage of tissue dry weight. Means ± 95% confidence limits.

TABLE 2. Concentrations of plasma and hemocyte metabolites in blood taken from scallops immediately after collection ("field") and 24 h later ("laboratory"). Plasma metabolites expressed as milligrams per 100 millilitres blood, hemocyte metabolites as milligrams per gram wet weight cells. Values given as mean ± SD. Means for field and laboratory determinations compared by *t*-test (self-pairing design).

Metabolite	Field	Laboratory	*t*	df	*P*
		Plasma			
Ammonia nitrogen	0.24 ± 0.12	0.18 ± 0.09	1.56	8	NS
Lowry positive	169 ± 27	160 ± 37	1.82	9	NS
Nonprotein Lowry	15.9 ± 3.2	14.8 ± 3.5	0.76	9	NS
Protein	153 ± 28	145 ± 36	1.46	9	NS
Nonprotein nitrogen	6.7 ± 1.2	7.1 ± 2.5	0.56	9	NS
Total carbohydrate	5.2 ± 1.2	5.3 ± 2.5	0.14	9	NS
Lipid	13.7 ± 2.4	12.4 ± 2.8	3.59	9	0.01
		Hemocyte			
Protein	49.2 ± 13.1	56.4 ± 11.9	1.05	9	NS
Low MW carbohydrate	0.60 ± 0.17	0.65 ± 0.15	0.62	9	NS
Glycogen	1.31 ± 0.35	1.49 ± 0.43	1.43	9	NS
Total carbohydrate	1.91 ± 0.46	2.13 ± 0.39	1.39	9	NS
Lipid	9.4 ± 1.4	10.6 ± 1.4	2.05	9	NS

carbohydrate were highest in August, decreasing considerably during the autumn and winter and rising from April to July. The quantity of glycogen present in the hemocytes was significantly greater in the summer than in the winter. During the winter, glycogen made up 65–70% of the carbohydrate in the blood cells, but for much of the summer the polysaccharide accounted for 80–85% of the total carbohydrate.

TABLE 3. Single classification analysis of variance for blood metabolite determinations. The least significant difference (critical difference) between means has been calculated where appropriate. *F* = variance ratio.

Metabolite	*F*	df	*P*	LSD ($P = 0.05$) mg·100 ml^{-1}
		Plasma		
Ammonia nitrogen	9.60	10, 48	< 0.001	0.17
Lowry positive	4.78	10, 55	< 0.001	21
Nonprotein Lowry	5.79	10, 37	< 0.001	6.1
Protein	4.14	10, 37	< 0.001	29
Lipid	8.32	10, 55	< 0.001	1.2
Low MW carbohydrate	9.85	10, 55	< 0.001	0.88
Glycogen	5.54	10, 53	< 0.001	1.4
Total carbohydrate	5.17	10, 53	< 0.001	1.7
Nonprotein nitrogen	2.59	10, 49	0.013	1.0
		Hemocyte		
Low MW carbohydrate	4.99	10, 48	< 0.001	0.14
Glycogen	3.36	10, 49	0.002	0.69
Total carbohydrate	3.38	10, 48	0.002	0.79
Lipid	1.20	10, 45	NS	—
Protein	1.38	10, 49	NS	—
Hematocrit	1.10	10, 41	NS	—

Associations Between Plasma Metabolite Concentrations

Spearman rank correlation coefficients were calculated for the plasma metabolite data, using all values obtained for each variable without regard for sampling date (Table 4). Significant associations were found between lipid concentration and concentrations of protein, low molecular weight carbohydrate, glycogen, and total carbohydrate. There was no association between low molecular weight carbohydrate and glycogen or protein levels, but concentrations of both glycogen and total carbohydrate were correlated with lipid and protein. All significant correlations were positive.

Size and Numbers of Hemocytes

Analyses of blood samples from 12 scallops collected in January were carried out using a Coulter counter and channelyzer:

Hemocyte count	11,500 cells·mm^{-3} ± 2700 SD
Modal volume	499 μm^3 ± 22 SE
Median volume	582 μm^3 ± 19 SE

A typical frequency distribution for cell volume, representing a scan of 178,000 hemocytes from a single blood sample (Fig. 8), shows a range of values for cell diameter similar to that given by Moore and Lowe (1977) for *M. edulis* hemocytes. A value of 0.74% was calculated for the hematocrit, using data presented above for cell numbers and median volume. The measured hematocrit in the same sample was 1.0% (mean value).

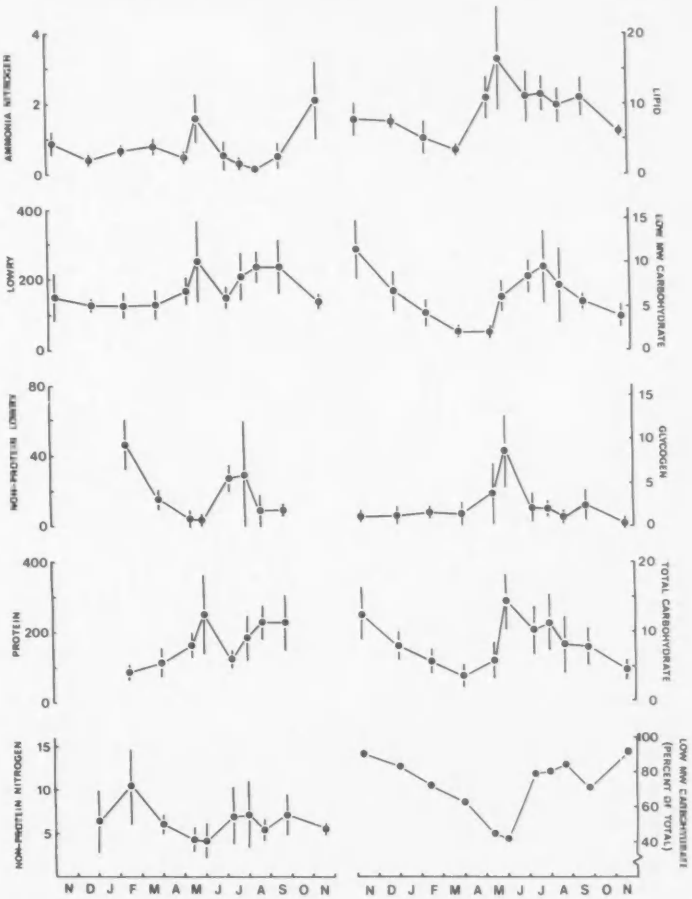

FIG. 6. Plasma metabolite levels (milligrams per 100 millilitres). Means ± 95% confidence limits.

COMPOSITION OF THE LIPID FRACTION IN THE PLASMA AND HEMOCYTES

Phospholipids and free sterols are the major components of plasma lipids, particularly during the summer (Fig. 9). During April and May, concentrations of diglycerides, triglycerides, and fatty acids increase, but these changes are relatively small when compared with the rises in plasma phospholipid and free sterol levels.

Free sterols and phospholipids also constitute the major part of the hemocyte lipid (Table 5). In addition, there is some monoglyceride, and other neutral lipids are occasionally present. No seasonal pattern is evident.

The residue remaining on the origin after separation of neutral lipids by thin-layer chromatography was shown to be phospholipid by development in the appropriate solvent systems (all the material then moved from the origin). The major phospholipid components of the plasma and hemocytes are phosphatidyl choline and phosphatidyl ethanolamine, together with some phosphatidyl serine. Shieh (1968) found a similar distribution of phospholipid species in the tissues of *P. magellanicus*.

Discussion

The reproductive cycle of *P. magellanicus* in Newfoundland is similar to that described by Mason (1958) for *Pecten maximus* around the Isle of Man and by Ansell (1974) for *C. septemradiata* in the Clyde Sea. Gonad maturation occurs in early summer and spawning takes place during August and September. Populations of

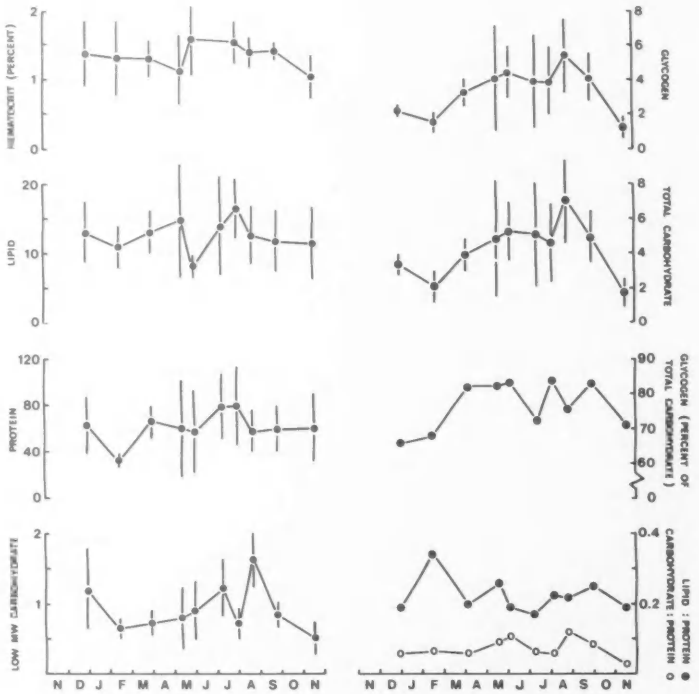
HEMATOCRIT (PERCENT)
GLYCOGEN
LIPID
TOTAL CARBOHYDRATE
PROTEIN
GLYCOGEN (PERCENT OF TOTAL CARBOHYDRATE)
LOW MW CARBOHYDRATE
LIPID : PROTEIN ●
CARBOHYDRATE : PROTEIN ○
N D J F M A M J J A S O N
N D J F M A M J J A S O N

FIG. 7. Hemocyte metabolite levels (milligrams per 100 millilitres blood). Means ± 95% confidence limits.

TABLE 4. Test for associations between plasma metabolite concentrations. Upper right of matrix — Spearman rank correlation coefficients with significance levels in parentheses; lower left, degrees of freedom.

Metabolite	Ammonia nitrogen	Nonprotein nitrogen	Protein	Lipid	Low MW carbohydrate	Glycogen	Total carbohydrate
Ammonia nitrogen	—	−0.258 (NS)	−0.195 (NS)	−0.199 (NS)	−0.183 (NS)	−0.001 (NS)	−0.061 (NS)
Nonprotein nitrogen	53	—	−0.217 (NS)	−0.244 (NS)	0.168 (NS)	−0.045 (NS)	−0.031 (NS)
Protein	42	48	—	0.475 (<0.001)	0.246 (NS)	0.329 (0.024)	0.515 (<0.001)
Lipid	59	60	48	—	0.311 (0.011)	0.446 (<0.001)	0.466 (<0.001)
Low MW carbohydrate	59	60	48	66	—	−0.043 (NS)	0.743 (<0.001)
Glycogen	57	58	47	64	64	—	0.495 (<0.001)
Total carbohydrate	57	58	47	64	64	64	—

Placopecten magellanicus from the Bay of Fundy and the New England coast also spawn in late summer (Dickie 1955). In *P. magellanicus* and *C. septemradiata*, spawning is followed by a nonreproductive phase which lasts throughout the winter. However, in *Pecten maximus* from the Clyde Sea, gonad differentiation and proliferation occur during the winter, and the scallops spawn in June and July (Comely 1974). Several species of *Chlamys* in Manx waters also spawn in late summer (Reddiah 1962). An earlier, minor spawning is common in most of these pectinids,

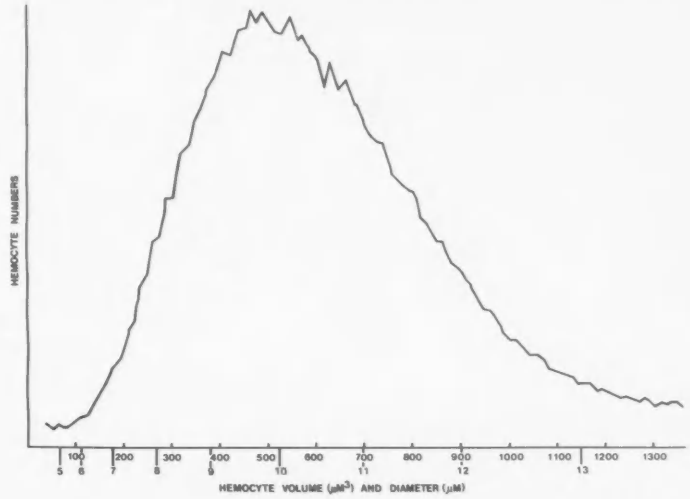

FIG. 8. Frequency distribution for hemocyte volumes, obtained from a Coulter channelyzer analysis of a single blood sample.

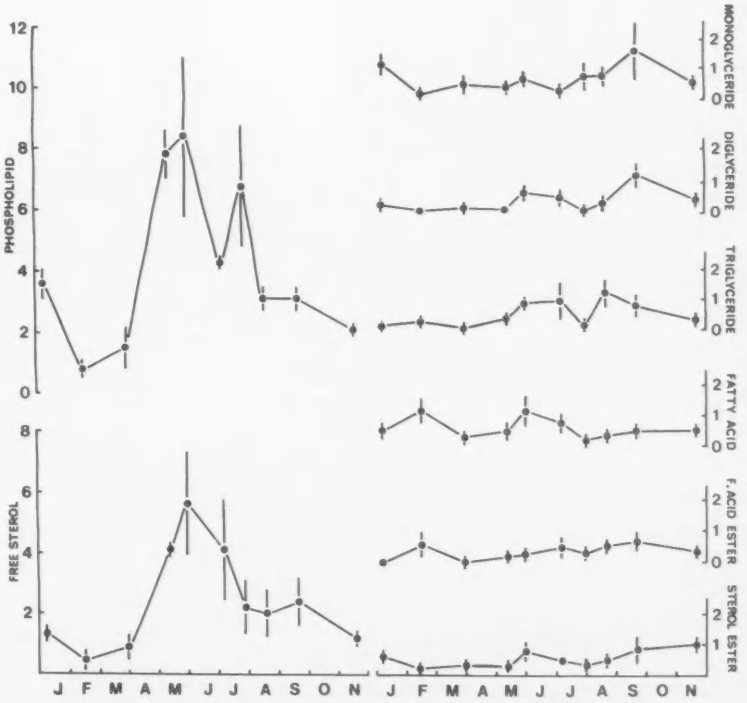

FIG. 9. Components of the lipid fraction from plasma, plotted on a common scale for ease of comparison. Means ± SD.

TABLE 5. Composition of the lipid fraction in hemocytes. Each component is expressed as a percentage of the total lipid present in the cells.

Date	Phospholipid	Mono-glyceride	Sterol	Diglyceride	Fatty acid	Triglyceride	Fatty acid ester	Sterol ester
Feb. 18, 1976	59.5± 2.7	12.3±2.6	28.2±2.1	—	—	—	—	—
Mar. 29, 1976	36.2± 5.7	5.3±3.8	52.8±8.6	—	—	—	—	4.1±4.9
May 11, 1976	64.4± 6.5	6.3±4.5	22.0±4.7	3.9±3.1	1.6±3.3	1.6±2.1	—	—
July 7, 1976	63.8± 9.3	1.9±2.2	21.2±4.1	—	16.0±10.8	—	—	—
Aug. 19, 1976	42.7±10.8	9.7±2.6	34.3±3.7	—	9.1±3.4	—	—	1.6±0.8
Nov. 17, 1976	44.3± 5.5	11.3±6.8	43.9±2.0	—	—	—	—	—

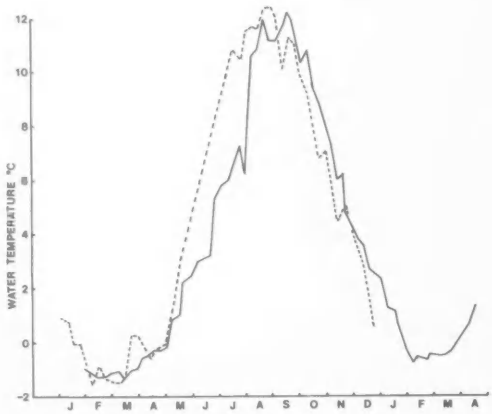

FIG. 10. Water temperatures at Logy Bay, Newfoundland, in 1972 (– – – –) and 1974–1975 (——). Courtesy of R. P. Scaplen (Memorial University, Newfoundland).

including *Placopecten magellanicus* from Port au Port Bay, Newfoundland (Naidu 1970).

During April and May, gonad and somatic tissues of *P. magellanicus* begin to increase in weight and in carbohydrate and lipid content. This occurs when the ambient water temperature is still low (0 to −1°C, Fig. 10), but food availability is high, due to the phytoplankton bloom which takes place in this area between March and May (Thompson and Buggeln unpublished data). Thompson et al. (1974) recorded similar increases in the digestive glands of mussels from a North Sea population during the spring bloom. In *P. magellanicus* the DNA content of the male gonad is also high by June, so that much of the differentiation precedes the temperature rise which occurs between June and August. Although differentiation and the accumulation of carbohydrate and lipid are initiated at low temperature, the gonad continues to increase in weight as the summer progresses and water temperatures rise. Almost all the material present in the ripe gonad is released in the gametes at spawning, and in late September the gonad enters the "resting" phase. At this time water temperature is at a maximum (approximately 12°C in eastern Newfoundland). In the Bay of Fundy, Dickie (1955) also found that *P. magellanicus* spawns when the water is warmest (usually 9–12°C) so that the larvae develop at optimum temperatures. Gonad growth in the bay scallop, *Aequipecten irradians*, also coincides with increasing temperature and peak phytoplankton production in early summer (Sastry 1966). Supplementary food is necessary for gonad development to continue in the laboratory, since reserves in the digestive gland alone are insufficient (Sastry 1968).

The most detailed descriptions of the relationships between energy reserves and the reproductive cycle in a marine bivalve relate to populations of the blue mussel, *M. edulis*, in eastern and southwestern England (Gabbott and Bayne 1973; Gabbott 1976). Gametogenesis takes place in winter, and the metabolic demands of this process are met primarily by utilization of reserves synthesized during the previous summer and autumn. In *P. magellanicus*, energy reserves from the previous year do not appear to play a significant role in subsequent growth and maturation of the gonad. There is certainly no accumulation of reserves in the scallop gonad after spawning, whereas in the mussel large quantities of glycogen are synthesized in the gonad (mantle) and somatic (nonmantle) tissue for use during the early

phases of gametogenesis. Glycogen reserves never accumulate in the gonad of *P. magellanicus*; the carbohydrate level in the ripe gonad is the same as that in the spawned eggs (Thompson unpublished data), and the carbohydrate content is extremely low after spawning. Protein and lipid are catabolized during September and October in the somatic tissue of *P. magellanicus*, and there is some loss of carbohydrate during winter (to very low values in March), but these losses are relatively low compared with those in *M. edulis* undergoing gametogenesis in winter. In *C. septemradiata*, glycogen is mobilized from the adductor muscle during the winter, when differentiation of the gonad is beginning (Ansell 1974). In the adductor muscle of *Pecten maximus* from the Clyde Sea, glycogen is synthesized during the summer and reaches a maximum in November. This glycogen is utilized during the winter, particularly in November and December, presumably to meet the demands of gametogenesis, which occurs at this time (Comely 1974). The cycle in this population of *P. maximus* is therefore similar to that of *M. edulis* in eastern England.

There is no evidence to suggest that gonad maturation in *Placopecten magellanicus* is inhibited by low temperatures per se. Differentiation occurs at 0 to −1°C, and there is a considerable amount of gonad growth at temperatures below 2°C. In *M. edulis* from the North Sea, gametogenesis continues despite temperatures as low as 3°C (Bayne and Thompson 1970), yet in a similar environment the European oyster, *Ostrea edulis*, does not begin gonad development until the spring. In a review of reproductive cycles in bivalves, Bayne (1976) provides further examples of species in which gametogenesis does not proceed in the winter and discusses the relationships between the cycles of nutrient storage and those of gamete production. These interactions are often complex and may show differences between populations of a given species. In Newfoundland, for example, the reproductive cycle of *M. edulis* is similar to that of *P. magellanicus* (Thompson unpublished data). Gametogenesis takes place in early summer and is completed relatively quickly, whereas in *M. edulis* from Europe this phase of the cycle takes place in the winter and is more prolonged.

The levels of lipid, protein, low molecular weight carbohydrate, and glycogen in the plasma increase considerably between April and June. The glycogen content of the circulating hemocytes also rises during this period and remains high throughout the summer. The demands of an actively growing gonad in the vitellogenic phase (together with the relative abundance of food at this time) are probably responsible for some of these increases in blood metabolite concentrations. Since gonad development occurs without a significant contribution from stored energy reserves, increases in plasma lipid and protein are to be expected during this phase of the reproductive cycle; the substantial increase in the carbohydrate content of the somatic tissue during early summer may account for a high proportion of the sugar and glycogen in circulation, because the egg of *P. magellanicus* contains very little carbohydrate (Thompson unpublished data).

The considerable individual variation in concentrations of many plasma metabolites suggests that there is poor regulation of these levels, but the positive associations between several pairs indicate that the levels of certain metabolites are related and therefore predictable to some extent. For example, if the carbohydrate concentration in the plasma of an individual scallop is low, so too is plasma lipid. The variations observed between individuals sampled simultaneously may be due primarily to differences in the nutritional states of the scallops.

Glycogen constitutes at least 70–85% of the carbohydrate in scallop hemocytes. On the basis of histochemical observations, Moore (personal communication, the Institute for Marine Environmental Research, Plymouth, U.K.) estimates that the hemocyte population contains <2% of the total glycogen present in *M. edulis*. The corresponding figure in *P. magellanicus* is approximately 0.1% (if we assume that hemolymph makes up half the tissue wet weight; Martin et al. 1958), but this is an underestimate, because only circulating hemocytes were counted. Although the blood cells are concerned in the transport of glycogen, they do not represent a significant store of the polysaccharide.

Most of the increase in the quantity of lipid circulating in the plasma during the summer comprises phospholipids and free sterols. It is likely that much of this lipid is incorporated into the gametes. Idler et al. (1964) recorded a decrease in the sterol content of *P. magellanicus* adductor muscle from June to August and suggested that sterols were being transported to the developing gonad. In a review of the literature relating to energy reserves in the eggs and larvae of bivalves, Gabbott (1976) comments on the high phospholipid content of the egg and suggests that phospholipids contribute to the energy metabolism of the early larva. Hemocytes may also be involved in the transfer of phospholipid to the maturing gametes. Moore and Lowe (1977) observed granulocytes in close association with de-

veloping and mature gametes in *M. edulis* and demonstrated histochemically the presence of phospholipid in the gametes and in granules within the associated hemocytes. The high phospholipid content of *P. magellanicus* hemocytes is consistent with these observations, but there is nothing in this study to suggest that hemocyte phospholipid is transferred to the developing gametes, since no clear seasonal pattern is evident in the phospholipid content of the hemocytes, and no distinction is made between membrane bound phospholipid and phospholipid present in granules. Further work is necessary to elucidate the roles of plasma and hemocytes in the transport of lipids and other nutrients to the developing gonad in bivalve molluscs.

Acknowledgments

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Nutrient Distribution in the St. Lawrence Estuary[1]

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GREISMAN, P., AND G. INGRAM. 1977. Nutrient distribution in the St. Lawrence estuary. J. Fish. Res. Board Can. 34: 2117–2123.

The horizontal distribution of nutrients in the St. Lawrence estuary during early July of 1975 is presented. The major sources of nutrients to the estuarine system appeared to be from the St. Lawrence River and the intermediate layer of the Laurentian Channel. By the assumption of simple mixing and conservation, the surface distribution of nutrients has been calculated. Differences between the calculated and observed values may be attributable to consumption, variability of the freshwater source and of the internal tide amplitude.

Key words: nutrient distribution, estuarine circulation, mixing

GREISMAN, P., AND G. INGRAM. 1977. Nutrient distribution in the St. Lawrence estuary. J. Fish. Res. Board Can. 34: 2117–2123.

Les auteurs décrivent la distribution horizontale des éléments nutritifs dans l'estuaire du Saint-Laurent au début de juillet 1975. Les éléments nutritifs entrant dans le système estuarien semblent provenir en grande partie du fleuve Saint-Laurent et de la couche intermédiaire du chenal Laurentien. La distribution superficielle des éléments nutritifs a été calculée en se fondant sur l'hypothèse de simple mélange et de conservation. Les différences entre les valeurs calculées et les valeurs observées peuvent être attribuables à la consommation et à la variabilité de la source d'eau douce et de l'amplitude de la marée interne.

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ON the basis of geographical and hydrographical consideration, the St. Lawrence system can be divided into four regimes: the Gulf of St. Lawrence seaward of Pointe des Monts; the maritime estuary between Tadoussac and Pointe des Monts; the middle estuary between Tadoussac and the toe of the salt wedge in the region of Île aux Coudres; and the fluvial St. Lawrence River which is nearly salt free, yet experiences tidal effects up to Lac St. Pierre (Fig. 1). The most striking bathymetric feature of the maritime estuary is the Laurentian Channel, with depths exceeding 300 m. This deep channel serves as a path for the intrusion of continental shelf and slope waters to its head, located near Tadoussac. Upstream of the maritime estuary, the river exceeds 50 m in depth only in a few areas.

The salinity of the system varies from $< 0.1‰$ in Lac St. Pierre to over $33‰$ at depths > 150 m in the Laurentian Channel. Isohalines along a longitudinal section of the estuary from Île d'Orléans to Pointe des Monts are shown in Fig. 2 from data collected on the C.S.S. *Dawson* in late June of 1975. Although the stations used in constructing this section were taken randomly over the tidal cycle, they illustrate the gross features of the salinity distribution. Strong salinity gradients are present in the salt wedge near Île aux Coudres and at the confluence of the Saguenay and St. Lawrence rivers, near Tadoussac. Also apparent near Tadoussac, at the shoaling of the Laurentian Channel, is an "upwelling" of deep water, as indicated by the anomalously shallow depth of the $33‰$ isohaline. The presence of a large semidiurnal internal progressive tide in the maritime estuary (Forrester 1974) suggests that this isohaline shoaling is a periodic phenomenon. The horizontal tidal excursion is usually < 10 km.

The general trend of increasing productivity toward the head of the Laurentian Channel, and surface waters that appeared to be enriched in nutrients, led Steven (1974) to hypothesize a "nutrient pump" near the confluence of the St. Lawrence and Saguenay rivers. This region was considered to be the most important source of nutrients for the estuary and the Gulf. Steven thought that the river's contribution of nutrients

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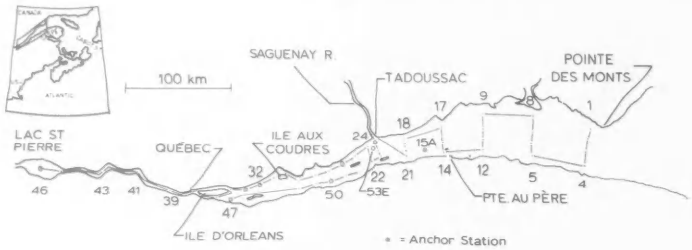

FIG. 1. Stations sampled, as well as anchor stations, over a complete semidiurnal tide cycle. Numbering sequence of stations starts at Pointe des Monts and increases upstream. Station 21 was expanded to four stations: 21A, B, C, and D.

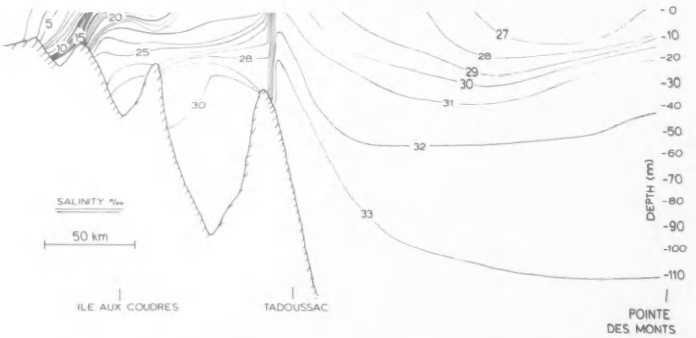

FIG. 2. Isohaline distribution for a longitudinal section constructed from all nonanchor stations along the northern shore (i.e. stations 1, 8, 9, 17, 18, 21B, 24, 25, etc.).

to the system was unimportant, since the freshwater of the St. Lawrence River is greatly diluted by seawater in the upper estuary between Île d'Orléans and Tadoussac. The nitrate-nitrite concentrations in the deep water at the Laurentian Channel are high, in excess of 20 μg-at (atoms)/ℓ throughout the year (Coote and Hiltz 1975).

As a result of the emphasis placed by Steven on the "nutrient pump" mechanism, several studies attempting to quantify the biological and physical processes in the Saguenay–St. Lawrence confluence area were undertaken in the region during 1973 and 1974 (Ingram 1975; Therriault and Lacroix 1976). The upwelling and mixing of intermediate depth water at the mouth of the Saguenay was attributed by Greisman and Ingram (1975) to shear instabilities in the internal tide waves.

Between June 26 and July 6, of 1975, during a cruise on C.S.S. *Dawson*, salinity, temperature, and nutrient concentration were measured in the area between Pointe des Monts and Lac St. Pierre. The station locations are shown in Fig. 1. At the anchor stations, data were recorded, including current-velocity profiles, over a semidiurnal tidal cycle. A Bendix Q-9 current meter was employed for profiling, and STD observations were made with a Guideline instrument. Nutrient samples were frozen and analyzed ashore with a Technicon Auto Analyzer. Although phosphate and silicate concentrations were also measured, we shall only discuss the nitrate–nitrite nitrogen concentrations, since Steven (1974) identified this nutrient as limiting the primary production. At each anchor station, profiles of all variables were made approximately once per hour.

In this paper we shall compare the relative importance of the freshwater nutrient sources with those originating in the maritime estuary. By the assumption of simple mixing, the distribution of surface nutrients along the estuary has been calculated. Differences between these values and the observed ones are used to indicate regions of nutrient uptake and other sources.

Observed Nutrient Distribution

In Fig. 3, isolines of nitrate–nitrite nitrogen (N) are shown on a map of the St. Lawrence

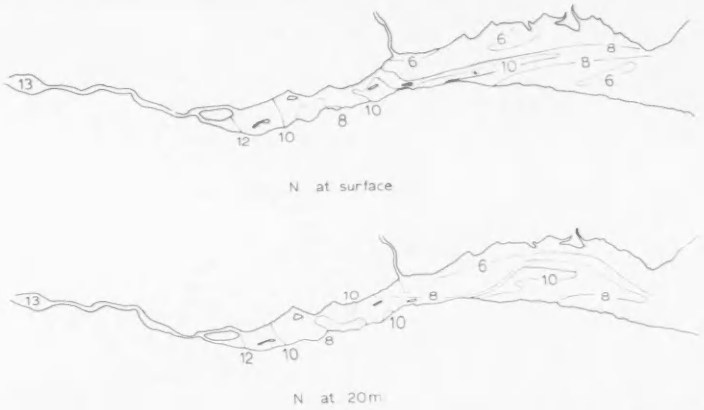

FIG. 3. Horizontal patterns of nutrient concentration (N) in microgram-atoms per litre constructed from nonanchor station results.

estuary system. As in Fig. 2, the plotted values are not synoptic and were taken randomly over the tidal cycle. The largest values of N in the surface layer are found far upstream, within the freshwater part of the system. Downstream of Île aux Coudres, N values range between 6 and 10 μg-at/ℓ. Below a depth of 20 m, significantly larger values of N are found only in the deep waters of the Laurentian Channel. This can be readily seen in the nutrient–salinity plot shown in Fig. 4, which includes data from all of the nonanchor stations, at varying depths throughout the water column. Similar to Coote and Hiltz (1975), values of N exceeding 20 μg-at/ℓ are found only at great depth and are associated with salinities of about 34‰. These two figures suggest that there are only two major sources of nutrient supply to the euphotic zone in the estuary: the freshwater river and the deep water of the Laurentian Channel. In the remainder of the paper we shall attempt to determine the relative importance of these sources.

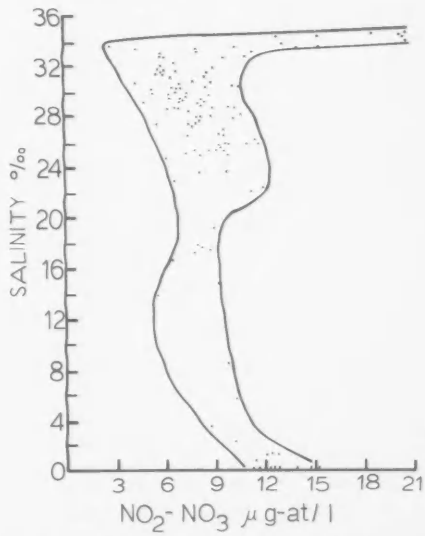

FIG. 4. Nutrient salinity plot of all observations taken at nonanchor stations during the 1975 cruise.

Calculated Nutrient Distribution

To identify the water mass that contributes nutrients to the euphotic zone through the agency of upwelling, we plotted a time dependent T–S diagram from data collected over a semidiurnal tidal cycle at anchor station 21B (Fig. 5, *upper*). This station is located within the upwelling zone at the head of the Laurentian Channel. The largest salinity values in the surface layer occurred at approximately high tide, in agreement with the findings of Hachey et al. (1956). For comparative purposes, a T–S plot was also constructed from data taken seaward of the upwelling zone, at station 18 (Fig. 5, *lower*). At this deeper location, we can clearly see a water mass at intermediate depth that is characterized by a temperature minimum. Although the depth of this layer varies to some degree over the tidal cycle, as a result of the internal tide, its average depth is approximately 75 m (Ingram 1975). These plots demonstrate that the upwelling water found at station 21B is characterized by T–S properties found in the temperature minimum layer at about 80 m depth. Although data taken at stations 18 and 21B are subject to tidal period vertical oscillations of the density field, the marked isohaline shoaling apparent in Fig. 2 occurs only at the head of the Laurentian Channel (station 21B).

To examine the nutrient concentration as a

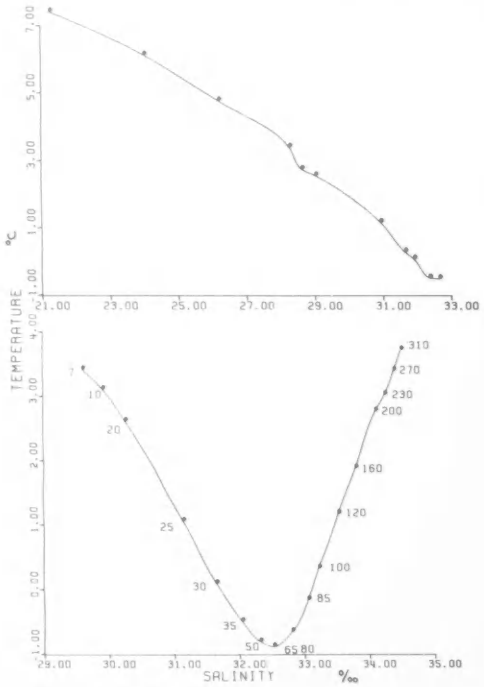

FIG. 5. T–S plots. Upper: values observed in upper 10 m at Station 21B over a semidiurnal cycle. Lower: values observed in single STD profile at station 18; depth of each point is shown in metres.

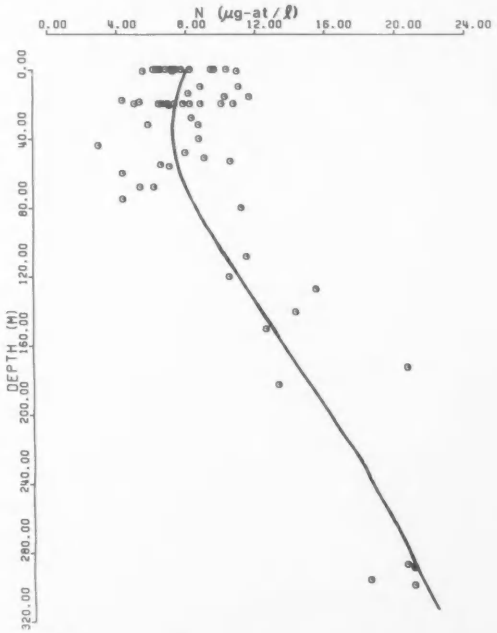

FIG. 6. Plot of all nutrient observations vs. depth taken at the nonanchor stations seaward of the Île Rouge Sill.

function of depth for the area seaward of the shoaling region (near Tadoussac) we plotted data from all of our stations in this region (Fig. 6). This plot reveals that waters of the temperature minimum layer and shallower depths have N values comparable to the surface layer, about 8 μg-at/ℓ, but significantly lower than those found in Lac St. Pierre.

To determine the relative importance of river-borne and upwelled nutrients we plotted the expected longitudinal distribution of nutrients resulting from these two sources, using the fractional freshwater method developed by Ketchum (1955). We assumed that the freshwater source was characterized by an N value of 13 μg-at/ℓ, while the upwelling source was taken to have a salinity of 32.5‰ and an N value of 8 μg-at/ℓ. In Fig. 7 the variation of salinity in the upper 10 m (depth-averaged) and the N contribution from the freshwater source, N_F, and from the upwelled estuarine source, N_E, are plotted. The two contributions are related to the salinity by the following expressions:

$$N_F(x) = N_R\left(\frac{S_L - S_{OBS}(x)}{S_L}\right)$$

$$N_E(x) = N_L\left(\frac{S_{OBS}(x)}{S_L}\right)$$

where $N_F \equiv$ the longitudinally varying concentration of N originating in the freshwater of the river; $N_R \equiv$ N in Lac St. Pierre, assumed to be characteristic of the undiluted river contribution (13 μg-at/ℓ); $N_E \equiv$ the longitudinally varying concentration of N originating in the temperature minimum layer and upwelled to the surface by estuarine tidal processes; $N_L \equiv$ the N concentration in the temperature minimum layer of the Laurentian Channel (assumed to be 8 μg-at/ℓ); $S_L \equiv$ the average salinity of the temperature minimum layer (assumed to be 32.5‰); $S_{OBS} \equiv$ the observed salinity; and $x =$ distance along the estuary, with its origin in Lac St. Pierre.

The relative importance of each nutrient input decreases with distance from its source (Fig. 7). In the upper reaches of the estuary the river-borne nutrients predominate in importance. The two sources contribute equally in a region some 25 km downstream of Île aux Coudres. In the

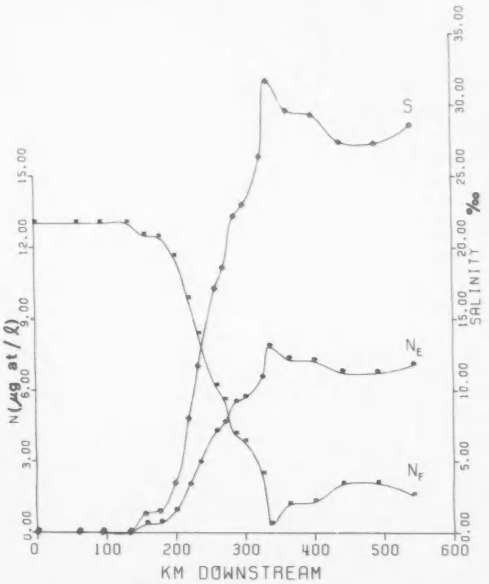

FIG. 7. Calculated nutrient distribution from each of two sources, origin of abscissa is station 46.

maritime estuary, slightly less than one quarter of the nutrients in the surface layer originated in the river upstream of Île d'Orléans.

By summing the contribution from the two nutrient sources, N_E and N_F, we are able to estimate the nutrient distribution that would be present in the surface layer if there were no consumption by phytoplankton and no other nutrient inputs. Figure 8 shows the calculated and observed nutrient concentrations along the river–estuary system. The differences between the calculated and observed values may be attributed to nutrient uptake by phytoplankton and to other sources, both man-made (pollution) and natural (such as nutrient regeneration). Considering the marked nonconservative nature of nutrient distributions in most estuaries (Peterson et al. 1975) it is surprising that the estimated values rarely differ by more than 2 μg-at/ℓ from the observed concentrations.

Nutrient Fluxes

To examine the reliability of the above estimates using alternative techniques, we decided to compute the advective nutrient flux in the river and the maritime estuary. The seaward nutrient flux from Lac St. Pierre is taken to be the product of N in Lac St. Pierre and the average river discharge, which is $\sim 10^4 m^3 \cdot s^{-1}$. The river thus supplies 13 μg-at/ℓ $\times$ $10^4 m^3 \cdot s^{-1}$ or 130 g-at$\cdot s^{-1}$ to the estuarine system. The nutrient flux typical of a downstream estuarine location seaward of the upwelling zone was computed at station 15A, using the following method. The speed and direction records were decomposed into seaward and cross-channel components with positive directions chosen downstream and towards the north shore. The N value at a specific time and depth was then paired with a value of the seaward velocity component. Plots of the product of seaward velocity component U, and N vs. time were constructed for one semidiurnal tidal cycle at each sampling depth. The net seaward flux of nitrate-nitrite nitrogen past the anchor station per unit current width is

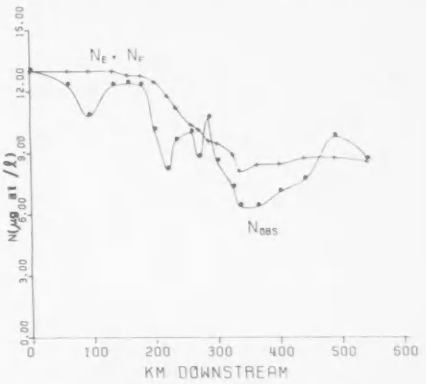

FIG. 8. Comparison of calculated and observed nutrient distribution.

$$\frac{1}{T}\int_0^T \int_0^d U(t, z)\, \mathrm{N}(t, z)\, dz\, dt$$

where T is the lunar-semidiurnal period, d is the local depth, z is the vertical coordinate, and t is time. The integral was evaluated graphically from the plots. The resulting flux value includes the contribution from both the mean and fluctuating components of the velocity and N fields. This concept can be better visualized by decomposing the instantaneous measurements of the velocity and N concentration into a mean and fluctuating component:

$$U = \overline{U} + U'$$
$$\mathrm{N} = \overline{\mathrm{N}} + \mathrm{N}'$$

where U and N are the measured hourly values, the overbars denote mean values over the tide cycle and the primes denote deviations from the

mean. Multiplication yields

$$U\,N = \overline{U}\,\overline{N} + U'\,\overline{N} + \overline{U}\,N' + U'\,N'$$

and the average of U N over a tidal cycle is

$$\overline{U\,N} = \overline{U}\,\overline{N} + \overline{U'\,N'}$$

since $\overline{U'} = 0$ and $\overline{N}' = 0$. Both components of the flux at stations in the St. Lawrence estuary are necessary to obtain reasonable estimates. The product $\overline{U}\ \overline{N}$, as computed by Sinclair et al. (1976) when calculating fluxes in the lower maritime estuary, is an adequate estimate of the total flux only when the time variations of U and N are orthogonal. Results obtained by Therriault and Lacroix (1976) suggest that these variables do not generally satisfy this condition.

The value of the total flux per unit current width at station 15A is 44.1 mg at·m^{-1}·s^{-1}. If this value is assumed to apply across a current whose width is 15 km, then the total nitrate–nitrite nitrogen flux in the lower estuary is 660 g-at·s^{-1} or about 5 times the flux at Lac St. Pierre. This result is in good agreement with the computed mixing ratios. River-derived nutrients are apparently responsible for roughly one fifth of the nutrients introduced into the euphotic zone in the maritime estuary.

Discussion

During the time of our observations periodic upwelling at the head of the Laurentian Channel was limited to the upper 80 m or so of the water column (Fig. 5). The water mass found between a depth of 70 and 80 m is characterized by a temperature minimum (usually below zero), salinities of ~32.5‰, and N values of about 8 μg-at/ℓ. Water characteristic of the deeper nutrient rich layer of the maritime estuary was not found within 10 m of the surface.

By the assumption of simple mixing and nutrient concentration behaving as a conserved property in the estuary, we have calculated the theoretical N distribution along the channel of the St. Laurence estuary from Lac St. Pierre to Pointe des Monts. We assumed that there were only two sources of nutrients: the freshwater river and the periodic upwelling near Tadoussac. The largest surface nutrient values are found in the freshwater source. As a result of dilution this supply represents the largest proportion of the total nutrient concentration only as far as 25 km downstream of Île aux Coudres. Seaward of Tadoussac, the diluted freshwater nutrients represent $< 25\%$ of the total concentration. This is not an insignificant contribution, as originally suggested by Steven (1974). Calculations of the advective nutrient flux in Lac St. Pierre and the maritime estuary support the findings.

By comparing the calculated and observed nutrient distribution with downchannel distance we found rather good agreement (Fig. 8). One might expect the observed nutrient concentrations to be generally lower than those calculated assuming conservation. With the exception of two regions this relationship was observed. The most striking "anomaly" was found in the lower maritime estuary, where the results were suggestive of other sources, rather than nutrient uptake. Although Fig. 8 was constructed from data taken along the central axis of the Laurentian Channel, one can readily see a plume of high-nutrient water to the south of this axis in Fig. 3. It is this plume that causes the anomalous response in Fig. 8 (near the 500-km point). These elevated values cannot be accounted for using our simple two source model. If we exclude nutrient regeneration and pollution from the argument, there are three possible explanations for the larger concentrations. The first is simply another source in the maritime estuary, such as the three north shore rivers in the downstream region. Secondly, one must consider the seasonal variability of the freshwater source which will manifest itself as a geographical variability at downchannel locations. Jordan (1973) has found the St. Lawrence to have maximum discharge in the latter half of April. As this is the time of maximum land drainage, we might expect more elevated nutrient concentrations in the freshwater source during this period. If the mean speed of the surface layer is 6 cm/s, it would take approximately 2 mo for the influence of this nutrient maximum to reach the maritime estuary. The suggested speed is not an unreasonable estimate. The third possibility is the variability of the upwelling source. At the time of our observations the amplitude of the surface tide was small (neap) and thus we might expect a reduction in the height of the baroclinic oscillations (Forrester 1974). If water from depths > 100 m was upwelled to the surface layer during spring tides, this would manifest itself in a geographical variability resulting from the fortnightly oscillations of the estuarine nutrient source.

In general, estimating the distribution of nutrients within an estuarine environment is a complicated exercise because of the numerous sources and sinks that result in temporal and spatial variability. By assuming only two sources of nutrient in the St. Lawrence estuary and simple mixing we have determined the theoretical proportion of the total N from the freshwater river and from the intermediate layer of the Lauren-

tian Channel. On the basis of the good agreement found between the observed and predicted nutrient distribution, the suggestion by Steven (1974) of a "nutrient pump" at the head of the Laurentian Channel is verified by our findings. The importance of river supplied nutrients is not negligible. A comparison of the observed and calculated concentrations can be used to designate areas of larger nutrient uptake.

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Responses of a Fish Population Model to Young-of-the-Year Mortality[1]

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DeAngelis, D. L., S. W. Christensen, and A. G. Clark. 1977. Responses of a fish population model to young-of-the-year mortality. J. Fish. Res. Board Can. 34: 2124–2132.

A multiple-age-class model is used to examine the effects of increases in density-independent young-of-the-year mortality caused by power plant entrainment of larval fish. It is demonstrated analytically that in all realistic cases, an increase in such mortality results in a smaller equilibrium population density of adult fish. The stability of the population with respect to perturbations about its equilibrium point is increased in these cases. However, situations can occur where a slight increase in mortality causes a catastrophic population decline. The model is used to generate autoregression graphs of population numbers that can be compared with field data.

Key words: stock–recruitment, young-of-the-year mortality, compensation, Leslie matrix, mathematical model

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Nous examinons, à l'aide d'un modèle à classes d'âge multiples, les effets d'augmentations, indépendantes de la densité, de la mortalité des jeunes de l'année résultant de la prise de poissons larvaires dans une centrale hydro-électrique. On démontre analytiquement que, dans tous les cas réalistes, une telle augmentation de la mortalité entraine une plus faible densité d'équilibre de population de poissons adultes. Dans ces cas, la stabilité de la population par rapport aux perturbations autour de son point d'équilibre augmente. Cependant, il peut se présenter des cas où une légère augmentation de la mortalité produit un déclin catastrophique de la population. Nous utilisons le modèle pour produire des graphiques d'autorégression de nombres de populations qui peuvent être comparés aux données sur le terrain.

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The use of stock–recruitment theory for predicting the effects of fishing exploitation on fish stock densities is so well established that it is natural to apply these same methods towards assessing other possible human impacts on fisheries. Increased mortality of immature fish could be as great a concern to the maintenance of fish populations as overfishing of adults. The entrainment of zero age-class or young-of-the-year (y-o-y) fish by the cooling systems of power plants is one such source of mortality. The question of the ultimate effects of power-plant-induced mortality on the fish stock has been receiving considerable attention recently (Jensen 1971; Van Winkle et al. 1974, 1977; Christensen et al. 1976, 1977) and warrants further work, especially in view of the current debate concerning the degree to which y-o-y fish mortality may be mitigated by compensatory mechanisms.

The application of traditional stock–recruitment theory to predicting the effects of increased y-o-y mortality poses the question of whether these methods can be adapted to situations where a fair amount of realism is demanded. The approaches of Beverton and Holt (1957) and Ricker (1954) consist of dividing the fish population into stock (reproducing fish) and recruits (the immature fish which will constitute the reproducing population at the next time step). This lumping of fish into only two classes, sexually mature and immature, makes impossible the inclusion of more detailed age-dependent characteristics. Impacts such as power-plant entrainment are strongly age dependent. More detailed age-structure models based on the Leslie matrix approach (Jensen 1971; Sommani 1972; Allen and Basasibwaki 1974; Beland 1974; Lawler 1974; Van Winkle et al. 1974; Hess et al. 1975; McFadden 1976; Vaughan and Saila 1976) are a major improvement in accurately representing fish populations. However, from the point of view of understanding the effects of compensatory mechanisms on

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y-o-y survival, these models suffer from two general inconveniences. First, Leslie matrices are discrete time-interval models working with yearly intervals. In contrast, the essence of compensatory mechanisms is that mortality rates are constantly changing in response to changing fish densities. Consequently, yearly intervals are much too large to permit analysis of anticipated short time-scale responses to density changes. Second, the modeling of mortality in the zero age-class is usually not detailed enough, or linked closely enough to actual biological phenomena, to allow for assessment of particular impacts.

Christensen et al. (1977) have introduced a model that attempts to alleviate both problems. The dynamics within age-classes are described by differential equations, and hence compensatory effects can be more appropriately modeled for the y-o-y fish. These differential equations are solved analytically, resulting in a simple Leslie matrix formulation. Furthermore, the principal types of mortality are modeled in detail for y-o-y fish, permitting convenient analysis of such impacts as power plant entrainment. In this paper several very general properties of the model are described with respect to the influence on the total fish population of changes in man-induced mortality to y-o-y fish.

The Model

The equations for a fish population divided into $N + 1$ age-classes are (Christensen et al. 1977),

$$\frac{dx_0(t)}{dt} = -(d_0 + g_0 x_0(t))\, x_0(t) - \sum_{i=1}^{N} \times c_{0i} x_i(t)\, x_0(t) \quad (1a)$$

$$\frac{dx_i(t)}{dt} = -d_i x_i(t) \quad (i = 1, N) \quad (1b)$$

where $x_i(t)$ is the numerical density of the ith age-class as a function of time and $i = 0$ denotes the zero (y-o-y) age-class. The parameter d_i is the rate of density-independent mortality, g_0 is the mortality coefficient of the y-o-y fish due to intra-age-class competition and/or cannibalism, and c_{0i} is the mortality coefficient of y-o-y fish due to cannibalism by or competition with older age-classes of the same species. The coefficients g_0 and c_{0i} would be classed, according to Ricker's (1954) definition, as rates involving compensatory mortality. The term "compensatory" is meant to imply that this type of mortality diminishes with decreasing values of population density. For example, if pressure on the y-o-y fish from background predation by other species, adverse physical conditions, or power plant entrainment increases, this acts through the mortality coefficient d_0, tending to lower the population density. However, this increased density-independent mortality also tends to decrease the mortality rates, $g_0 x_0(t)$ and $\sum_{i=1}^{N} c_{0i} x_i(t)$, partially compensating for the increase in d_0. The net decrease in the population is not as great as it would be without the compensation. Whether there are circumstances in which an increase in d_0 might, through overcompensation, lead to an increase in equilibrium population, is a question which is answered negatively for our model in subsequent analysis.

The biological reasoning behind these equations is also discussed by Christensen et al. (1977). The assumption of the operation of compensation mechanisms within the zero age-class, but not in the older age-classes, is in accord with Ricker (1954, 1975), Backiel and LeCren (1967), Cushing (1974), and others.

If a given spawning time is defined to occur at time $t = 0$, then a new cohort is formed at this time. The number of eggs constituting this new cohort is given by

$$x_0(0) = \sum_{i=1}^{N} f_i x_i(0) \quad (2)$$

where the f_i's are fecundity rates, in egg per fish, calculated from values of eggs per female and percentages of females in the various age classes. Also, at spawning time each of the older cohorts advances from one age-class to the next,

$$x_i(0) \to x_{i+1}(0) \quad (i = 0, N - 1) \quad (3)$$

The assumption, nearly always justifiable, that the mortality rate of the zero age-class is much larger than that of the older age-classes, permits obtaining an approximate analytic solution to the above system of equations. Given an initial set of age-class densities at the spawning time, $t = 0$, the densities at the next spawning time, $t = 1$, are given by

$$x_1(1) = \frac{(d_0 + C_0)\, F \exp(-(d_0 + C_0))}{d_0 + C_0 + g_0 F(1 - \exp(-(d_0 + C_0)))} \quad (4a)$$

$$x_{i+1}(1) = \exp(-d_i)\, x_i(0) \quad (i = 1, N - 1) \quad (4b)$$

where

$$F = \sum_{i=1}^{N} f_i x_i(0) \quad (5a)$$

$$C_0 = \sum_{i=1}^{N} c_{0i} \bar{x}_i \quad (5b)$$

and where $\bar{x}_i$ is the average value of $\bar{x}_i(t)$ in the

interval from $t = 0$ to $t = 1$:

$$\bar{x}_i = x_i(0)\,(1 - \exp(-d_i))/d_i \qquad (6)$$

Expression (6) is derived by solving equation (1b) for $x_i(t)$, averaging over a year by integrating from 0 to 1 and dividing by 1 (a year). In Christensen et al. (1977), $\bar{x}_i$ was simply set equal to $x_i(0)$, which is a reasonable approximation if adult age-class population do not decrease too much during the course of the year. Equation (4a) is obtained by integrating equation (1a) with the assumption that $x_i(t) = \bar{x}_i$, and then employing expression (3). The integration procedure is identical to the method used for integrating the logistic differential equation.

Equations (4a, b) constitute the relation between the vector of population densities at $t = 0$, $\mathbf{x}(0) = (x_1(0), x_2(0), \ldots, x_N(0))$, and the vector at $t = 1$, $\mathbf{x}(1) = (x_1(1), x_2(1), \ldots, x_N(1))$. This relation can be written as follows:

$$\mathbf{x}(1) = \mathbf{A}(x(0))\cdot\mathbf{x}(0) \qquad (7)$$

where $\mathbf{A}(\mathbf{x}(0))$ is an $N \times N$ density-dependent Leslie matrix. Therefore, the model has reduced to a classical form.

The model presented above is oversimplified from many points of view. Since it is a one-species model, feedback effects from changes in densities of various prey and predator populations are not taken into account. Furthermore, equations (1a, b) might not always accurately reflect the true population dynamics if for example, density dependent mechanisms exist in older age-classes. The approximation of $\bar{x}_i(t)$ by x_i over the time interval from 0 to 1, permitting the integration of equation (1a), also introduces some error. It is possible, if desired, to reduce this error source by dividing the 1st yr of life into a number of subintervals, for each of which an equation like (1a) is solved. An appropriate average value is substituted for $x_i(t)$ in each of these subintervals. In this way a much more accurate, though more complicated, solution of the system of equations can easily be obtained analytically. Alternatively, equations (1a) and (1b) could be solved numerically, by computer, eliminating the need for approximating $x_i(t)$. However, numerical solutions lack the advantage of generality afforded by an analytic solution. For the following analysis, it is convenient to restrict discussion to equations (4a) and (4b), but the results are expected to apply to the more general version of the model as well.

Population Density Equilibria

Once the model parameters have been estimated using measured population data where possible, the model can be used to predict the effects of external perturbations on a fish population. Satisfactory validation of the model for particular parameter values is still remote because of the time and difficulty involved in obtaining necessary data. However, it is possible to deduce certain conclusions concerning the model's behavior which are very general and fairly independent of particular parameter values. To begin with, it is interesting to consider the response of the population equilibria to changes in the rate of y-o-y density-independent mortality, d_0, which is likely to increase when an environmental impact directly affects age-class 0.

Because all natural fish populations are buffeted by external forces, no population ever stays precisely at its equilibrium point, even if this point is theoretically stable. Nevertheless, the average values of the age-class densities, over many years, will be close to the equilibrium point if the point is stable. The equilibrium point for the present model is most easily found by first expressing all densities in the right-hand side of equation (4a) in terms of $x_1(1 - j)$, $(j = 1, N)$, which can be done by applying equation (4b) repetitively. Hence, $x_2(0) = \exp(-d_1)x_1(-1)$, $x_3(0) = \exp(-d_1-d_2)x_1(-2)$, etc. For example, F then becomes

$$F = \sum_{j=1}^{N} f_j x_1(1 - j) \exp\left(-\sum_{k=1}^{j=1} d_k\right) \qquad (8)$$

Next, equilibrium is assumed, implying

$$x_1(0) = x_1(-1) = \ldots = x_1(1 - N) \qquad (9)$$

that is, the population density of the first age-class is the same from year to year. When this assumption is used in equation (4a) and $x_1(1)$ is plotted against $x_1(0)$, a curve with the typical form shown by the two curves in Fig. 1 results. This curve resembles the Ricker-type curve of simple stock–recruitment theory, although it is not a classical stock–recruitment curve, since the abcissa represents only one older age-class, not all adults. If $c_{0i} = 0$, $(i = 1,N)$, then the curve would rise asymptotically to a plateau like the Beverton–Holt curve (see Beverton and Holt 1957, p. 51), rather than reaching a maximum and then declining. The equilibrium point lies at E_1, the intersection of this curve and the 45° line,

$$x_1(1) = x_1(0) \qquad (10)$$

The equilibrium values of the remaining age-class densities can be found using equation (4b).

The parameter values used in Fig. 1 are listed in Table 1 and Fig. 1. These mortality coefficients and fecundities are not implausible for a population of striped bass in an estuary. Of the values

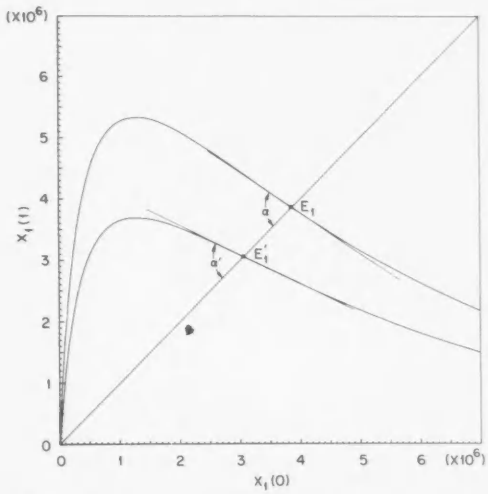


FIG. 1. Equilibrium points, E_1 and E_1', of the model for different values of y-o-y mortality, d_0. The axes $x_1(0)$ and $x_1(1)$ are the population numbers of age-class 1 in years 0 and 1, respectively. The equilibrium value of x_1 decreases (i.e. $E_1' < E_1$) as d_0 increases. Also, the slope at the equilibrium point, E_1 becomes less negative (i.e. $S_E' > S_E$, where $S_E' = -\sin(\alpha' - 45°)$ and $S_E = -\sin(\alpha - 45°)$); $d_0 = 9.0$ for the upper curve and $d_0 = 9.4$ for the lower curve. The other parameter values are given in Table 1.

TABLE 1. Parameter values relevant to Fig. 1, where f_i has units of (number of egg)·(number of fish of ith age-class)$^{-1}$, d_i has units of (year)$^{-1}$, g_i has units of (number of y-o-y fish)$^{-1}$·(year)$^{-1}$, and c_{0i} has units of (number of yearling fish)$^{-1}$·(year)$^{-1}$.

Age-class	f_i	d_i	g_i	c_{0i}
0	0.0	9.0	0.12×10^{-9}	0.0
1	0.0	0.92	0.0	0.0
2	0.0	0.51	0.0	0.20×10^{-6}
3	0.0	0.37	0.0	0.20×10^{-6}
4	0.0	0.56	0.0	0.20×10^{-6}
5	390,000.0	0.56	0.0	0.20×10^{-6}
6	325,000.0	0.56	0.0	0.0
7	415,000.0	0.45	0.0	0.0
8	685,000.0	0.45	0.0	0.0
9	770,000.0	0.37	0.0	0.0
10	905,000.0	0.37	0.0	0.0
11	885,000.0	0.32	0.0	0.0
12	885,000.0	0.32	0.0	0.0
13	885,000.0	0.28	0.0	0.0
14	885,000.0	0.28	0.0	0.0
15	885,000.0	0.28	0.0	0.0

of c_{0i}, only c_{02}, c_{03}, c_{04}, and c_{05} are nonzero, meaning that it is assumed here that only the 2-yr-old through 5-yr-old striped bass have a significant negative effect on the y-o-y fish.

By computational examination of the model, Christensen et al. (1977) found empirically that, when all other parameters were held constant, increases in d_0 were accompanied by decreases in the equilibrium value of $x_1(1)$ (and hence decreases in the equilibrium values of older age-classes as well) for a wide range of parameter values. This point can also be demonstrated analytically by considering small increments, δd_0 and $\delta x_1(1)$, in d_0 and $x_1(1)$, respectively. From the assumption of equilibrium, i.e. equations (9) and (10), it follows that $F = F'x_1(1)$; similarly, $C_0' = C_0'x_1(1)$. In fact, $x_1(1)$ can be written simply as x_1, since this quantity is independent of time. With the substitution of $x_1 + \delta x_1$ for x_1 and $d_0 + \delta d_0$ for d_0 in equation (4a), one quickly obtains

$$\{d_0 + \delta d_0 + C_0'(x_1 + \delta x_1) + g_0F'(x_1 + \delta x_1)\} \times (1.0 - \exp\{-d_0 - \delta d_0 - C_0'(x_1 + \delta x_1)\}) = \{d_0 + \delta d_0 + C_0'(x_1 + \delta x_1)\, F' \exp(-d_0 - \delta d_0 - C_0'(x_1 + \delta x_1)\} \quad (11)$$

Expansion of the exponents; i.e.

$$\exp\{-d_0 - \delta d_0 - C_0'(x_1 + \delta x_1)\} = \exp\{-d_0 - C_0\} \times \{1.0 - \delta d_0 - C_0'\,\delta x_1 + \tfrac{1}{2}(\delta d_0 + C_0'\delta x_1)^2 - \ldots\} \quad (12)$$

followed by the multiplication of factors in the above equation and the dropping of all terms $(\delta x_1)^i(\delta d_0)^j$ $(i + j > 1)$ results in

$$\delta x_1(1) = -(\eta/\xi)\,\delta d_0 \quad (13)$$

where

$$\eta = 1 + g_0F\exp(-(d_0 + C_0)) + F'(d_0 + C_0 - 1)\exp(-(d_0 + C_0)) \quad (14a)$$

$$\xi = C_0'\eta + g_0F'(1 - \exp(-(d_0 + C_0))) \quad (14b)$$

$$C_0' = \sum_{i=1}^{N} c_{0i}e_{i-1}(1 - \exp(-d_i))/d_i \quad (14c)$$

$$F' = \sum_{i=1}^{N} f_ie_{i-1} \quad (14d)$$

$$e_{i-1} = \exp(-\sum_{j=1}^{i-1} d_j) \quad (14e)$$

It is observed that η and ξ are always positive if

$$d_0 + C_0 > 1.0 \quad (15)$$

Condition (15) means that the probability of survival through age-class 0, considering both density-independent effect (d_0) and cannibalism and/or competition from older age classes (C_0), is < 0.37 $(= e^{-1})$. Since y-o-y fish usually suffer

extremely high mortality, this condition is expected to be true in all realistic cases, and one can conclude that $\delta x_1(1)$ (Eq. 13) will always be negative. Hence, even with compensation effects, there are no realistic circumstances in which an increase in d_0 can lead to an increase in the equilibrium value of $x_1(1)$.

The effects of the compensatory mortality rates g_0 and c_{0i} are apparent from equations (13) and (14). When c_{0i} and g_0 are increased this tends to cause $\delta x_1(1)$ to become less negative, because the magnitude of ξ is increased relative to η. For a given δd_0, there is then a smaller decrease in the equilibrium value, $x_1(1)$, with compensation, as expected.

Stability of Equilibria

There is no guarantee that the equilibrium point E_1 is stable, where we mean that E_1 is stable if it is locally "attracting" (May and Oster 1976). A small perturbation away from E_1 may grow into oscillations of increasing amplitude. These oscillations can be quite drastic, which may be an undesirable situation for a fishery. Even a very slight change in d_0, or one of the other parameters, might be sufficient to transform E_1 from a stable to an unstable equilibrium point.

A rigorous mathematical analysis to determine whether or not E_1 is stable is possible; Allen and Basasibwaki (1974) outlined the method, which employs eigenvalues. However, the procedure is quite complex algebraically, and if interest is only in the possible effect of a change, δd_0, in d_0 on the stability of E_1, a simpler alternative is preferable. It is possible to show that given a negative slope of the curve at E_1 in Fig. 1, the more negative the slope, the greater the chance that E_1 is unstable. (For positive slopes, E_1 is always stable.) Ricker (1954) pointed out that for the traditional stock–recruitment curve, to which the curve in Fig. 1 reduces when $N = 1$, E_1 is stable when the tangent to the slope lies between 0 and 1, and unstable when the slope lies between -1 and ∞, giving rise to oscillations in the latter case. For cases in which $N > 1$, the tangent value of -1 is no longer necessarily the demarcation point between stable and unstable behavior, but there will still be a trend towards greater instability as the slope becomes more negative.

It is possible to demonstrate that with other parameter values held constant, an increase of δd_0 in d_0, in addition to causing a decrease in the equilibrium value of $x_1(1)$ at E_1 (Eq. 13 and Fig. 1), nearly always causes an initially negative slope, S_E, at E_1 to become less negative (Fig. 1). That is, one finds,

$$\delta S_E = \kappa \delta d_0 \tag{16}$$

where κ is some positive quantity. To show this, both sides of equation (4a) are differentiated with respect to $x_1(0)$ to yield

$$S_E = \frac{dx_1(1)}{dx_1(0)} = C_0\left\{\frac{1.0 - g_0x_1(1)}{d_0 + C_0}\right\} - 1.0 + d_0/\{d_0 + C_0 + g_0F(1.0 - \exp\{-(d_0 + C_0)\})\} \tag{17}$$

Derivation of equation (17) requires considerable algebraic manipulation. Here, an indication of the procedure is given. Since the derivative S_E is taken at the equilibrium point, then $F = F'x_1(1) = F'x_1(0)$ and $C_0 = C_0'x_1(1) = C_0'x_0(0)$. Differentiating the right hand side of (4a) by the chain rule, one obtains

$$\frac{dx_1(1)}{dx_1(0)} = \frac{d}{dx_1(0)}\left\{\frac{\{d_0 + C_0'x_1(0)\}F'x_1(0)\exp\{-d_0 - C_0'x_1(0)\}}{d_0 + C_0'x_1(0) + g_0F'x_1(0)(1.0 - \exp\{-d_0 - C_0'x_1(0)\})}\right\}$$
$$= \frac{C_0'F'x_1(0)\exp\{-d_0 - C_0'x_1(0)\}}{d_0 + C_0'x_1(0) + g_0F'x_1(0)(1.0 - \exp\{-d_0 - C_0'x_1(0)\})} + \text{additional terms} \tag{18}$$

where the first term on the right hand side is the term resulting from differentiation of the factor $d_0 + C_0x_1(0)$. Using equation (4a), one obtains

$$\frac{dx_1(1)}{dx_1(0)} = \frac{C_0}{d_0 + C_0} + \text{additional terms} \tag{19}$$

The "additional terms" are treated in the same way as the first term on the right hand side to eventually obtain equation (17).

An expression for the change in slope, $\delta S_E = \delta[dx_1(1)/dx_1(0)]$ in terms of δd_0 is obtained by substituting $d_0 + \delta d_0$ and $x_1(0) + \delta x_1(0)$ for d_0 and $x_1(0)$, respectively, in the right-hand side of equation (17). Equation 13 is used to eliminate $\delta x_1(0)$ in favor of δd_0. The resulting expression for δS_E is very complicated, but somewhat simpler conditions can be extracted from it. If equation (15) is satisfied as well as the inequality

$$\frac{g_0F \exp(-(d_0 + C_0))}{1.0 + (g_0F/C_0)/(1.0 + g_0F \exp(-(d_0 + C_0)))}$$
$$+ \frac{d_0}{d_0 + C_0 + g_0F(1.0 - \exp(-(d_0 + C_0)))}$$
$$- S_E > C_0 \qquad (20)$$

then equation (16) is guaranteed to hold and therefore, the probability of the population being stable increases for a positive δd_0. The more negative the slope at equilibrium, S_E, and the smaller the rate of cannibalism, C_0, the more likely it is that equation (20) is satisfied. The condition, which is a rather conservative one, holds for nearly all realistic parameter values. This has been corroborated by directly plotting out $x_1(1)$ using equation (4a) for a wide range of parameter values in which negative slopes at equilibrium always became less negative when d_0 was increased.

If our fish population model is a reasonable approximation of reality, the analysis in this section indicates that increased density-independent mortality on y-o-y fish will almost always tend to increase the stability of the population since it causes the slope at E_1 to become less negative. However, power plants or other human impacts may affect fish populations in other, less direct ways than simply through increases in d_0. For example, interference with the prey species of the fish in question could lead to instability of the fish population. Also, the above result holds only for "stability" as it is defined here. There are other ways of characterizing stability which may be as important as the type of stability considered in this section. In the last section of this paper, another sort of stability, the stability against "catastrophic" types of population decline, is considered and shown to decrease as d_0 increases.

It is interesting to examine the types of oscillations which can occur when the fish population becomes unstable. Consider again a fish population having approximately the parameters in Table 1 except that we assume here that the y-o-y fish are cannibalized only by the yearling class (i.e., only $c_{01} \neq 0$). This could conceivably be the case in some fish populations (though perhaps not striped bass), and since the dynamic behavior of such a fish population is interesting, it is worth considering. Since there is little quantitative knowledge of the coefficient c_{01}, it is profitable to allow this parameter to take on different values. For relatively small values of c_{01} ($<10^{-6}$), $x_1(t)$ approaches a constant value with increasing time, meaning that the population is stable (Fig. 2, curve *a*). As c_{01} increases, the population can exhibit very complex oscillatory behavior (Fig. 2, curve *b*). The pictured oscillation is a 2-yr cycle modulated by a 22-yr cycle. The occurrence of this 22-yr cycle for $c_{01} = 0.15 \times 10^{-4}$ is surprising, but it can perhaps be explained. The strength of the cannibalism and competition from the yearling class of the y-o-y fish is enough to produce 2-yr oscillations which rapidly grow in amplitude. But when these fluctuating cohorts reach the fifth age-class, where spawning begins, this age-class affects the y-o-y numbers in a way that is out of phase with the effects of the yearling class. Years of large egg production coincide with years of high yearling cannibalism and competition pressure and vice versa. Hence the oscillations are damped, as seen in Fig. 2. When equilibrium is nearly reached the oscillations begin again. The magnitude of c_{01} determines the length of the long cycle; the larger c_{01} is, the longer this modulation cycle. If spawning started in the fourth or sixth age-class, this would reinforce the effects of the yearlings on the y-o-y population, possibly leading to violent oscillations. The important conclusion from these results is that very complex population behavior, including long cycles, is suggested from this very simple model. No examples of the "chaotic" behavior observed by May and Oster (1976) for simple stock–recruitment models were found in our limited samplings of parameter values, but it may be that this type of behavior will occur for large enough values of c_{01}.

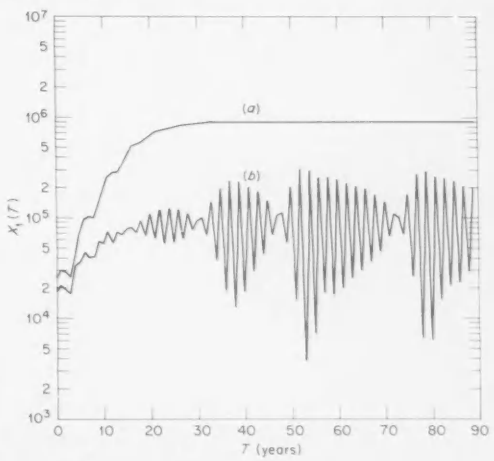


FIG. 2. Simulations of equations (4a, b) for two values of c_{01}, (a) 10^{-6} and (b) 0.15×10^{-4}, with $c_{0i} = 0.0$ ($i = 2, \ldots, 15$). The other parameter values are the same as in Table 1. $x_1(t)$ is the population number in age-class 1 as a function of time t.

The striped bass population characterized by the parameters of Table 1 does not exhibit oscillatory behavior, even when c_{02} through c_{05} are increase in magnitude. This is a result of the cannibalism on y-o-y fish being spread out among several older age-classes.

Autoregression Graphs

Plotting of relative fish population data from year to year, by using catch-per-unit-effort data or data on similar indices, is unlikely to reveal such regular behavior as that shown in either curve of Fig. 2. For the purpose of comparing the model with fisheries data, it may be more advantageous to look at autoregression graphs, with population densities of any of the age-classes at different years as axes.

Autoregression graphs can be generated by computer simulations of the model. To add realism it is possible to include some random fluctuations in the model parameter values. Using approximately the same set of parameters as used earlier in Fig. 2 and allowing the mortality coefficients (only the d_i's in this case) to vary randomly about mean values with standard deviations equal to 10% of the average values, a set of autoregression graphs was generated from the model. Figure 3 exhibits typical graphs of $x_1(0)$ vs. $x_1(j)$ for $j = 1, \ldots, 5$. A strong negative correlation is evident between $x_1(0)$ and $x_1(1)$, reflecting the negative influence of yearlings on y-o-y fish. On the other hand, $x_1(0)$ and $x_1(2)$ are positively correlated because a large value of $x_1(0)$ suppresses $x_1(1)$, due to increased cannibalism by $x_1(0)$ on $x_1(1)$. This allows a large value of $x_1(2)$ due to decreased suppression of $x_1(2)$ by $x_1(1)$. The correlations between $x_1(0)$ and $x_1(3)$ and $x_1(4)$ and $x_1(5)$ are much weaker.

The use of such autoregression graphs for comparing the model with fisheries data may help achieve better choice of parameters for the model. The discussion here only barely scratches the surface of this matter, however.

Catastrophic Behavior

Although it has been shown in the section on stability of equilibria that increases in d_0 tend to increase the stability of the population model, the analysis applies only to "local" stability, or stability in the vicinity of an existing equilibrium point. It does not rule out phenomena in which the equilibrium point itself disappears. This is demonstrated below.

The assumption in the model concerning the production of eggs, equation (2), is at best only a fair approximation of reality. In general, small population densities can be nonoptimal for survival or reproduction because of effects on social interaction. This is known as the "Allee effect" and has been noted in fish populations (Odum 1971). Instead of equation (2), one might assume

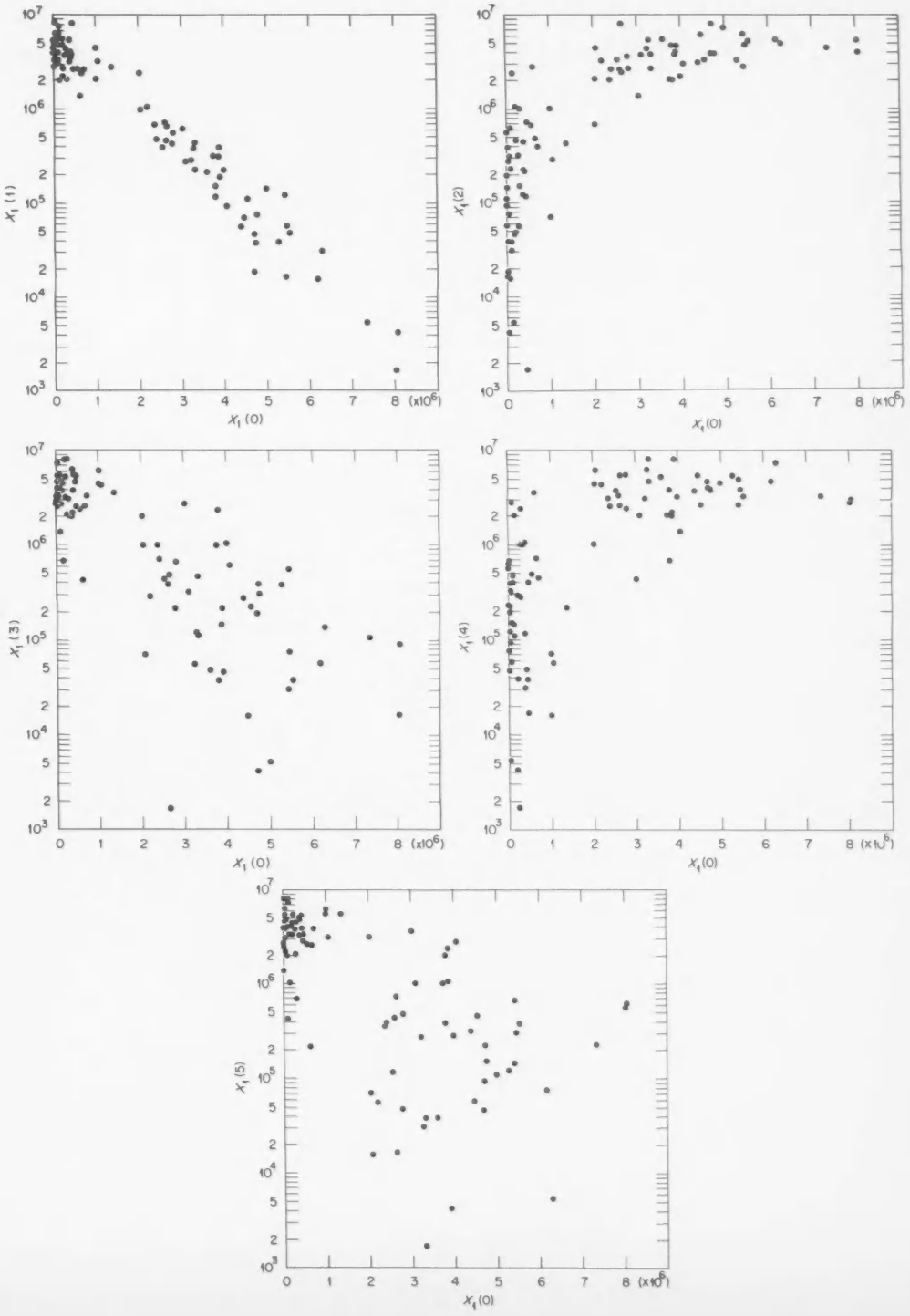
$$x_0(1) = \sum_{i=1}^{N} f_i x_i^{\gamma}(1)/(x_i^{\gamma-1}(1) + a_i) \quad (21)$$

where ($\gamma > 1$ and a_i) is a positive constant. For very small values of $x_i(1)$, $x_0(1) \sim x_i^{\gamma}(1)$, while for large $x_i(0)$ the form of equation (2) is approached. The curves shown in Fig. 1 now take the general form shown in Fig. 4, in which there are two equilibrium points, E_1 and E_2. Only E_1 can ever be stable. Population densities starting near E_2 either go to E_1 or tend toward extinction at the origin.

Consider an equilibrium point, E_1, assumed to be stable (Fig. 4). The parameter a_i in equation (21) is chosen so that the egg production per fish is reduced to 50% of its original level if the total fish population density is reduced to 10% of its equilibrium value. The choice of this value is arbitrary to make the phenomenon described below clearly visible in a graph. Suppose d_0 is gradually increased, slowly enough that the system stays close to its equilibrium point, E_1. As d_0 increases, the curve decreases in height and E_1 decreases gradually. However, a time is reached when E_1 and E_2 coalesce into a single point. Any further increase in d_0, no matter how small, causes the equilibrium point to vanish, as shown in the bottom curve in Fig. 4, and extinction of the modeled population follows. It can further be shown that even a quick decrease in d_0 may not be able to halt the population decline once it has started.

This type of behavior, with a very small change in a parameter, in this case d_0, causing a very pronounced effect, is related to the theory of bifurcating solutions (Minorsky 1962) and is also a simple case of a mathematical "catastrophe", the theory of which was developed by Thom (1975). Since theoretical examples of such catastrophes have been exhibited in nearly all areas of biological science, it is not surprising to see a case in fish population dynamics. Whether such

FIG. 3. Autoregression graphs for the population of age-class 1 at year $t = 0$, $x_1(0)$ vs. the population of age-class 1 at later years, $x_1(j)$. Parameter values are the same as in Fig. 2, except for random variations in the d_i's.


$X_1(1)$
$X_1(0)$
$X_1(2)$
$X_1(0)$
$X_1(3)$
$X_1(0)$
$X_1(4)$
$X_1(0)$
$X_1(5)$
$X_1(0)$
(x10^6)

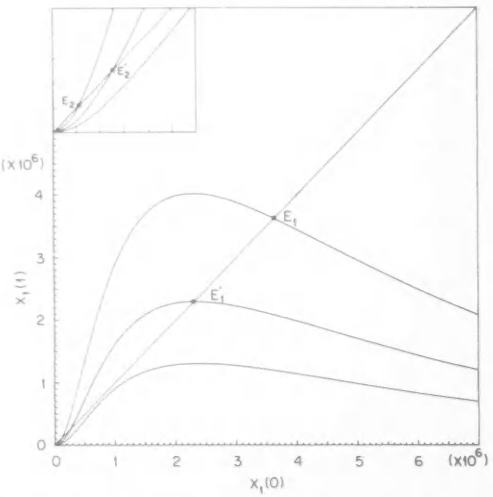


FIG. 4. Behavior of the stable equilibrium point, E_1, as d_0 increases. Aside from d_0, parameter values are the same as in Table 1, with the additional parameters $= 2.0$ and $a_1 = 100{,}000$. $d_0 = 9.0$, 9.6, and 10.2 in the upper, middle, and lower curves, respectively. E_2 is the unstable equilibrium point, $x_1(0)$ and $x_1(1)$ are the population numbers of age-class 1 in years 0 and 1, respectively. Inset enlarges lower left-hand corner.

dramatic events as predicted by the model can actually happen in nature is not certain, but Holling (1973) has suggested that some rapid, apparently irreversible changes in fish populations known to have occurred may be real-world examples of this type of mathematical behavior. The fact that such theoretical possibilities exist underscores the need for a deeper understanding of fish dynamics.

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Some Aspects of Benthic Production in a Salmonid Rearing Channel

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WILLIAMS, D. D., J. H. MUNDIE, AND D. E. MOUNCE. 1977. Some aspects of benthic production in a salmonid rearing channel. J. Fish. Res. Board Can. 34: 2133–2141.

Experiments on optimizing the use of benthos in a salmonid rearing channel are described. The cropping potential of invertebrates for use as fish food was measured by substrate recolonization which showed that 80,000 animals, weighing approximately 0.62 g (dry weight), could be liberated from 1 m^2 of clean gravel substrate to a depth of 15 cm every 15 days. Recolonization curves expressed in terms of number of taxa, total numbers, and biomass gave the following patterns: asymptotic regression curve, logistic growth curve, and a straight line, respectively. Individual curves of the major taxa showed a variety of patterns. Small accumulations of fish production wastes (up to 9 ℓ/m^2 of food pellets and up to 4.5 ℓ/m^2 of fish feces) increased benthic standing crop by as much as 3 times, but larger accumulations resulted in a decline together with a decrease in benthos diversity. We suggest that invertebrates ameliorate these potential pollutants through their feeding and living habits.

Key words: benthic production, salmon rearing, benthos recolonization, fish wastes

WILLIAMS, D. D., J. H. MUNDIE, AND D. E. MOUNCE. 1977. Some aspects of benthic production in a salmonid rearing channel. J. Fish. Res. Board Can. 34: 2133–2141.

Nous décrivons des expériences effectuées en vue de l'utilisation optimale du benthos dans un chenal d'élevage de salmonidés. Nous avons mesuré le potentiel de récolte d'invertébrés servant de nourriture aux poissons par le taux de recolonisation du substrat, qui démontre que 80 000 animaux, pesant environ 0.62 g (poids sec), pouvaient être libérés d'un substrat de gravier propre de 1 m^2 à une profondeur de 15 cm par 15 jours. Les courbes de recolonisation exprimées en termes de nombre de taxons, de nombres totaux et de biomasse ont les formes suivantes: courbe de régression asymptotique, courbe de croissance logistique et ligne droite, respectivement. Les courbes individuelles des principaux taxons ont diverses configurations. De faibles accumulations de déchets de production de poissons (jusqu'à 9 ℓ/m^2 de nourriture en boulettes et jusqu'à 4.5 ℓ/m^2 d'excréments de poisson) va jusqu'à tripler la biomasse benthique, mais de plus grandes accumulations entraînent une diminution de la biomasse et de la diversité du benthos. Nous suggérons que les invertébrés améliorent ces polluants possibles par leur régime alimentaire et leur mode de vie.

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MUNDIE (1974) examined the feasibility of raising salmon smolts in seminatural channels using mainly artificial foods. "This approach," he states, "should combine some desirable features of rivers with the productive capacity, but not the costs, of hatcheries." The role of the benthos would be to contribute to the diet of the fry and to scavenge on waste foodstuffs and feces. It is the potential of these two processes that is the concern of this present paper.

The Study Area

The work was carried out in a flow-control rearing channel, passing 0.42 m^3/s (15 cfs), constructed alongside the Big Qualicum River on the east side of Vancouver Island, B.C. The physical characteristics of the river are described by Lister and Walker (1966). Chemically the river is low in nutrients and has an alkalinity of 30 mg/ℓ $CaCO_3$. The features designed into the channel stress uniformity of conditions, so that phenomena such as edge effect, which play a major role in natural streams, have been virtually eliminated.

Experiment 1
Cropping Potential of Benthos for Fish Food

The importance of drifting benthic invertebrates in the diet of salmonid fry occurring at

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natural densities has been well documented, e.g. Mundie (1969). They frequently make up most of the diet. They provide vitamins and essential trace elements, and also a natural feeding experience which is usually denied to fish raised in artificial rearing facilities. As many of these organisms, however, drift only during darkness, fish are unable to make full use of them (Mundie 1971a). One method of increasing their availability to salmonids is by dislodging them artificially during daylight. This raises the question of how often an area of substrate may be disturbed without depleting benthic density and substantially lowering the food level. The following procedure was designed to examine this.

Materials and Methods

Eight wooden troughs each measuring 210 × 22 cm were laid side by side across the middle of a riffle at the lower end of the rearing channel. On January 5 each was lined with polyethylene sheet and filled with clean gravel (mean size 24.2 mm; range 38 mm) to a depth of 15 cm. The current flowing through the troughs was adjusted to approximately 45 cm/s by means of vertical slats at the upstream end. The substrates were then left to be colonized. This trough technique, with its standardized material and water velocities, was chosen as a means of imposing uniform conditions for the recolonization by drift.

After 3 days the contents of one of the troughs were raked and the drifting organisms collected in a 50-μm mesh net attached to the downstream end. One randomly chosen trough was similarly sampled every 3 days until the experiment ended on January 29. To ascertain the standing crops of benthos in the channel itself over this period, samples were taken from the channel gravel at the start (January 8) and after the end (February 16) of the experiment. These were taken with a 15-cm diam corer, similar to that used by Waters and Knapp (1961) but of all metal construction. This sampled an area of 177 cm² to a depth of 30 cm. The mesh of the collecting net was again 50 μm and three replicates were taken on both occasions. The temperature range during the period of the experiment was 4.7–5.3°C. All samples were preserved in 5% formalin. Later, they were sieved on a coarse screen and then subsampled to one-sixteenth units (Hynes 1961). For each sample, the animals were sorted from two units, one for identification and the other for dry weight (110°C for 24-h) estimates.

Results

Mean numbers of taxa in the channel were similar before (36.0) and after (34.7) the experiment. This level was reestablished after only 9 days. Even on day 3 there were 28 taxa present (Fig. 1A). A linear relationship exists between biomass and time ($P < 0.001$) (Fig. 1B). In terms of total numbers, the recolonization curve follows a logistic growth curve which levels off after about 15 days (Fig. 1C). Comparison of this level (approximately 80,000 animals /m²) with standing crop estimates in the channel reveals a threefold difference (January 8: $\bar{x} = 273{,}038$; February 16: $\bar{x} = 210{,}854/m^2$). The higher values in the stream were not unexpected, as the cores in the channel sampled to twice the depth of the gravel in the troughs, and recent studies (e.g. Williams and Hynes 1974) have shown that more than half the invertebrates in streams with gravel beds may occur below a depth of 15 cm. There was no significant relationship between trough contents and location across the riffle.

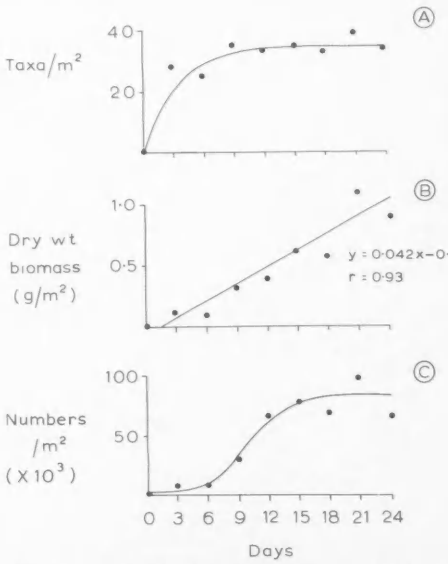

FIG. 1. Recolonization of benthos over 24 days: A) number of taxa, B) dry weight biomass, C) total numbers of animals per square metre. (The lines are least squares regressions selected by computer.)

Numerical recolonization curves for some individual taxa (Fig. 2–5) indicate that not all the species follow the general pattern seen in Fig. 1C. For example, the oligochaete *Nais variabilis* Piguet increases to a peak of around 400/m², followed by a decrease to around 150/m² (Fig. 2A). Figures 2B and D represent the curves for two species of zooplankton emanating from the lake of this stream's origin. The curves are similar and suggest that the two species' presence in the benthos was sporadic (and perhaps related to changes in discharge from the lake). The stonefly *Alloperla?elevata* Frison (Fig. 2C) tended to follow the general pattern of Fig. 1C. The

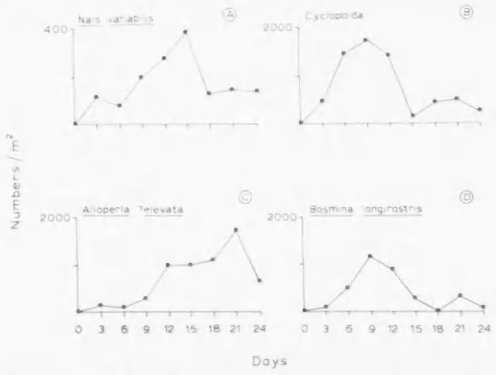

FIG. 2. Recolonization curves for various taxa. (Number of individuals of a species per square metre present at 3-day intervals over 24 days.)

two species of the mayfly genus *Baetis* possibly showed different rates of recolonization — *B. parvus* Dodds, gradual, while *Baetis* sp. A speeded up considerably during the 3rd wk (Fig. 3A, B). *Similium arcticum* Malloch and *Prosimulium* sp. (Fig. 3C, D) conformed more to the general pattern, as did the two species of the chironomid genus *Polypedilum* (Fig. 4A). The chironomid *Phaenopsectra*, on the other hand, appears to have overcolonized around day 12, after which its numbers declined to a level between 500 and 1000/m² (Fig. 4B). Again, the curves for the Tanytarsini genera *Micropsectra* (2 sp.) and *Rheotanytarsus* (Fig. 4C, D) seem to follow the general pattern, as do the four orthocladiines shown in Fig. 5. There are possible differences in the rates of recolonization prior to leveling off in the three species of *Eukiefferiella*.

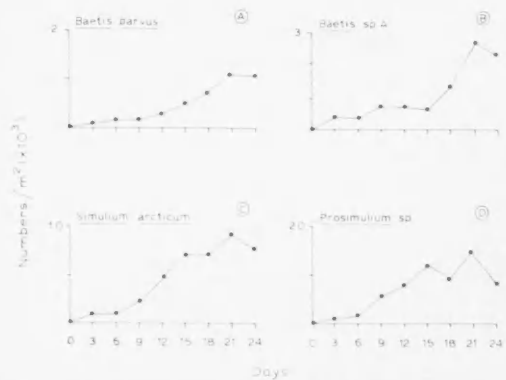

FIG. 3. Recolonization curves for two species of mayfly (A, B) and two species of blackfly (C, D).

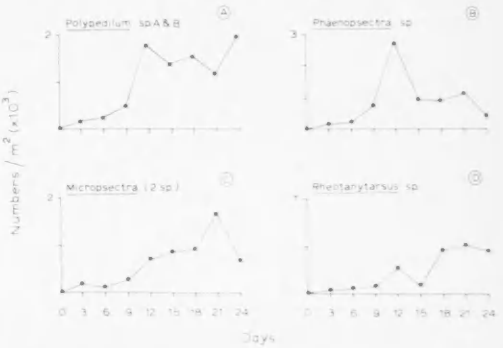

FIG. 4. Recolonization curves for two genera of Chironomini (A, B) and two genera of Tanytarsini (C, D).

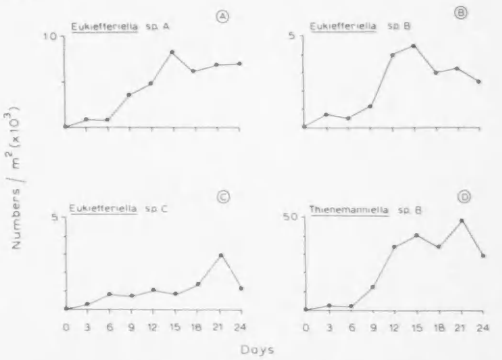

FIG. 5. Recolonization curves for four species of Orthocladiinae.

The core samples indicated a decline in numbers of some species in the channel over the period January 8 to February 16, e.g. *Thienemanniella* sp. B: $\bar{x}$ = 73,957 to 44,374/m². This presumably is the result of natural winter mortality.

Experiment 2
Response of Benthos to Waste Foodstuff

To be economically viable, rearing channels must support high densities of fish. Of necessity, most of their diet must be supplied artificially, usually in pellet form. These pellets contain protein, fats, and fiber (commonly derived from fish and animal viscera and grain by-products) in addition to carefully balanced vitamins and other supplements. Some brands float on the water surface for a time, while others sink. Tables giving recommended feeding rations are supplied so that food wastage is minimal, but pellets that are missed by the fish settle on the bottom and remain uneaten. They may accumu-

late to form a deposit many centimetres thick. Aquatic fungi colonize this material and form a cohesive mat. The effect that this has on benthos was examined as follows.

Materials and Methods

Eight wooden troughs were filled with gravel, in an identical manner to that in Experiment 1, and were left to be colonized in the Big Qualicum River during late July 1975. Two weeks later, food pellets were added randomly to six of the troughs in the following doses: 1, 2, 4, 6, 8, and 16 ℓ. The other two troughs were left clean and served as controls. The pellets used were O.M.P. brand (Oregon Moist Pellets, Formula II, manufactured by Moore-Clark Co., Washington), which is one of the sinking varieties used extensively on the west coast of North America. They were soaked in water for about 1 min before being poured evenly throughout the length of each trough. The troughs were then left for 4 wk, after which the contents were collected by raking each into a 50-μm drift net as in Experiment 1. Preserved material from each trough was subsampled into five equal units (Mundie 1971), three for counting and identification and two for dry weight biomass estimates. Each unit contained approximately 400 animals.

Results

The initial response is one of increasing numbers with increasing dosage to a point where 4 ℓ of pellets produced a 2.5-fold increase over the control density (Fig. 6A). An abrupt decrease occurred at 6 ℓ to a level only slightly greater than the controls. This density remains constant despite further additions. A similar pattern is evident for dry weight biomass (Fig. 6B), although the decline between 4 and 6 ℓ is not as pronounced. The number of taxa (Fig. 6C), however, shows a steady decline from the controls to a level established at about 4 ℓ. This decline is mainly attributable to the loss of the mayflies *Rithrogena* sp., *Cinygma* sp., and *Iron* sp., and the stoneflies *Nemoura* sp. and *?Acroneuria* sp. The loss of a few other minor taxa, such as the mites *Testudacarus minimus* Marshall and *Atractides* sp. and harpacticoids, also contributed to this decline.

Examination of some of the more important taxonomic groups, such as the Chironomidae (Fig. 7) reveals that numerically the subfamilies and some tribes show the same general response pattern as that of Fig. 6A, although the amplitude for the Tanypodinae, for example, is not as great. Response of individual species, however, are generally different. Figure 8 shows that whereas *Polypedilum* sp. B exhibits the general response, *Phaenopsectra* sp. peaks at a higher dose. Numbers of *Eukiefferiella* sp. A increase rapidly to the 4-ℓ level and then decline to twice the control density. *Cricotopus/Orthocladius* sp., on the other hand, is slow to respond at first but then peaks at 4 ℓ and levels out at approximately 20 times the control density.

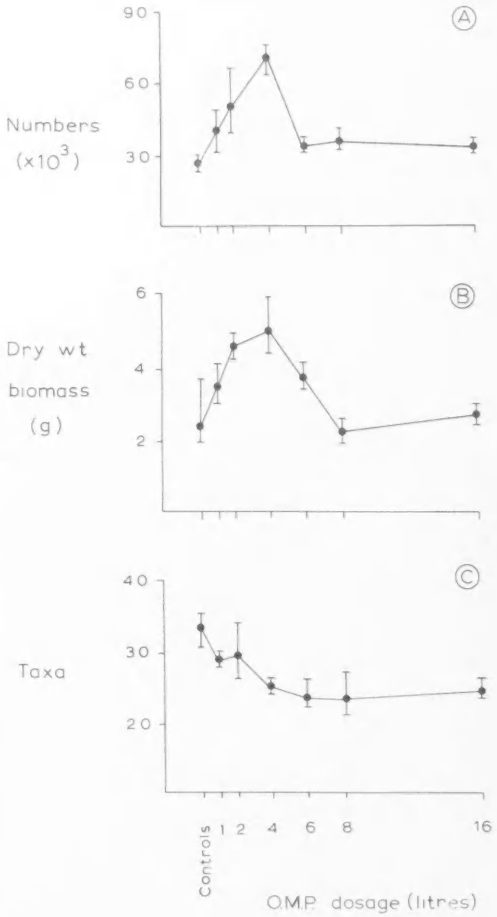

FIG. 6. Response of benthos to O.M.P. after 1 mo: A) total numbers of animals per trough, B) dry weight biomass, C) number of taxa. (Vertical lines show the range of the three subsamples.)

Experiment 3
Response of Benthos to Fish Feces

One of the major consequences of raising fish at high densities is that of accumulation of fish wastes. These wastes are voided in two basic forms, soluble and solid. Soluble products, such as ammonia and urea, the major nitrogenous

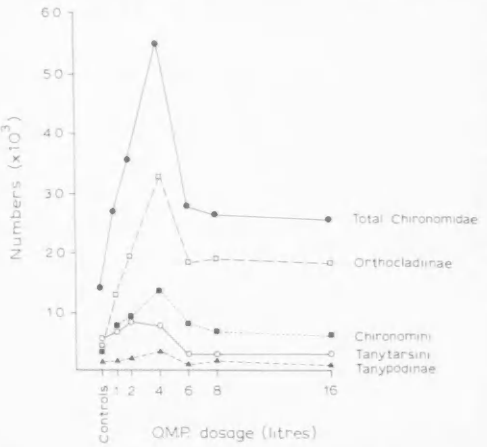

FIG. 7. Response of Chironomidae subfamilies to O.M.P. (Points represent means of the three subsample values.)

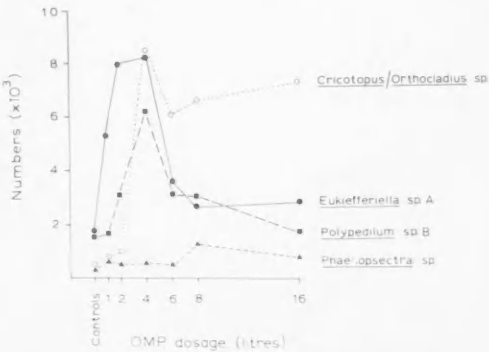

FIG. 8. Response of selected chironomid species to O.M.P.

wastes, are excreted from the gills and probably pass downstream. On the other hand, the solid material, consisting of indigestible fibers, inorganic particles, mucus, sloughed intestinal-epithelial cells, bacteria, and small amounts of amino acids, uric acid, creatine, and creatinine (Hickman and Trump 1969; and Brett 1977) voided through the gut, sink to the stream bottom. The effect of the accumulation of solid waste products on the fish themselves has been examined (Burrows 1964), but their effects on benthic invertebrates have not been well documented.

Materials and Methods

The eight wooden troughs previously used were set up in a riffle in the rearing channel, as in Experiment 1, and were allowed to be colonized. During early November 1975, when the water temperature was around 9°C, fish wastes were collected with a dip net from a nearby coho rearing facility. Large numbers of fish contained in this unit had, over a period of several months, produced a thick layer of wastes on the bed, consisting almost entirely of fecal pellets, although some O.M.P. and invertebrates were collected too. This mixture was allowed to settle in a bucket for 20 min, after which excess water was poured off. To maintain as fresh a mixture as possible, one quarter of the desired doses (i.e. 0.25, 0.5, 1.0, 1.5, 2.0, and 4.0 ℓ) was added to six troughs at the beginning of each week for 4 consecutive wk; the two remaining troughs served as controls. After 28 days the contents of each trough were collected and processed as described for Experiment 2.

Results

The numerical response of total benthos to increasing fish feces was similar to that for O.M.P., i.e. an initial enrichment effect, to a maximum of 2.3 times the control density (Fig. 9A). However, this peak occurred at 2 ℓ compared with 4 ℓ for O.M.P. Further, the subsequent decline is not as rapid and the plateau occurs at approximately twice control density. Biomass increases rapidly at first and then levels out at nearly 4 times the control (Fig. 9B). Species diversity also increases at first, probably as a result of some species having been added with the waste, but then declines gradually. This decline is primarily due to a loss from the fauna of the mayflies *Baetis* sp. A and *Cinygma* sp., the stonefly *Alloperla* ? *elevata* Frison, the blackfly *Prosimulium* sp., and cyclopoids and harpacticoids.

Detailed examination of the major taxa shows that chironomid larvae and oligochaetes (Naididae) predominate, with a change in dominance from 0.06:1 (oligochaete to chironomid) in the control troughs, to 1.88:1 in the 16-ℓ troughs (Fig. 10). Breakdown to chironomid subfamily or tribe responses (Fig. 11) does not show a similarity to the general pattern of total numbers (c.f. the O.M.P. experiment). The Tanytarsini, primarily *Rheotanytarsus* sp. and two species of *Micropsectra*, show an initial rapid increase followed by a gradual decline to a value below that of the control. The Chironomini show a steady increase with increasing dosage, while the Tanypodinae (*Procladius* sp., ?*Natarsia* sp. and a species from the *Thienemannimyia* group) show little change. The Orthocladiinae, on the other hand, show an unusual pattern with peaks at 2 and 8 ℓ, mainly, *Eukiefferiella* sp. A and B, *Thienemanniella* sp. B, and a major depression around 4–6 ℓ. More detailed examination of the chironomids (Fig. 12) reveals the following: *Brillia* sp., although present as a few large individuals in the

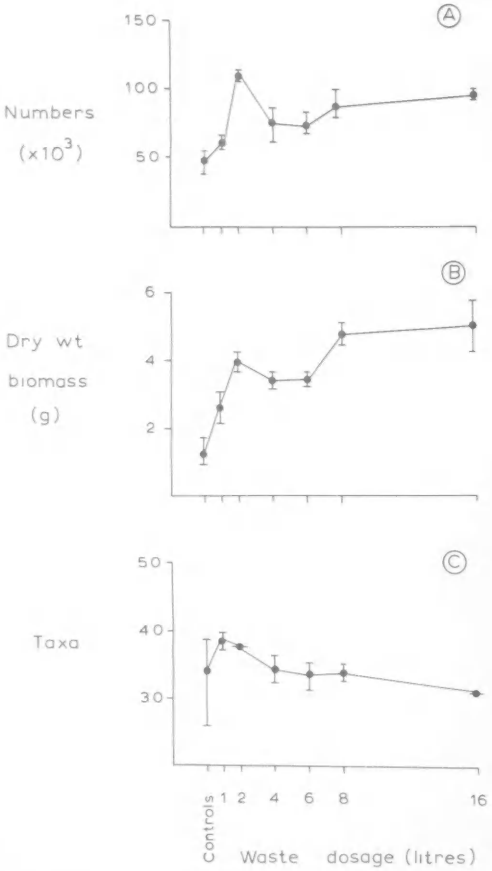

FIG. 9. Response of benthos to fish feces after 1 mo: A) total numbers of animals per trough, B) dry weight biomass, C) number of taxa. (Vertical lines show range of the three subsamples.)

FIG. 10. Ratio of Oligochaeta to Chironomidae in response to fish feces.

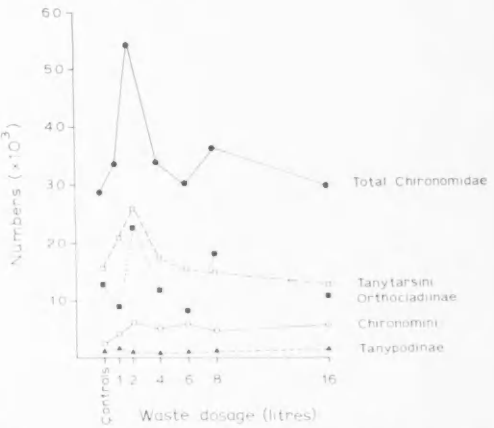

FIG. 11. Response of Chironomidae subfamilies to fish feces.

controls, establishes itself at about 700 animals/trough after the addition of only 1 ℓ of wastes and further additions have little effect; the overall steady increase of Chironomini is composed of a rapid influx of large *Pentapedilum* larvae starting at 4 ℓ, a gradual increase of *Phaenopsectra*, and a peaking of *Polypedilum* sp. A and B at 2 ℓ followed by their decline. Why there was a decline of Orthocladiines, as typified in Fig. 12 by *Eukiefferiella* sp. A, is unknown, unless it was a response to increased numbers of large predatory *Phaenopsectra* and *Pentapedilum* in the 6-ℓ trough.

The oligochaete fauna was dominated by three species (Fig. 13). Total numbers increased rapidly at first, but after 2 ℓ the rate of increase declined. *Dero digitata* (O. F. Müller) was the most abundant worm and followed this pattern, but *Chaetogaster diaphanus* (Gruithuisen) and *Nais variabilis* numbers soon leveled off at about 5000 individuals/trough. This suggests that some regulating factor was in effect, as worms were unavoidably added with the waste, and theoretically, their numbers should have kept increasing.

Discussion

The results of Experiment 1 show that natural benthic densities may be returned to small areas,

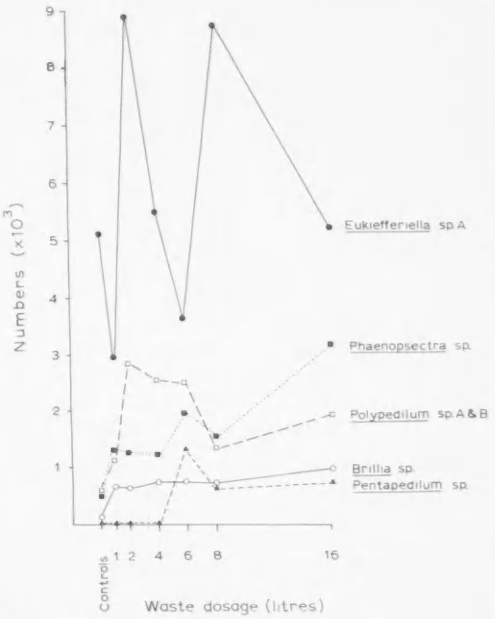

FIG. 12. Response of selected chironomid species to fish feces.

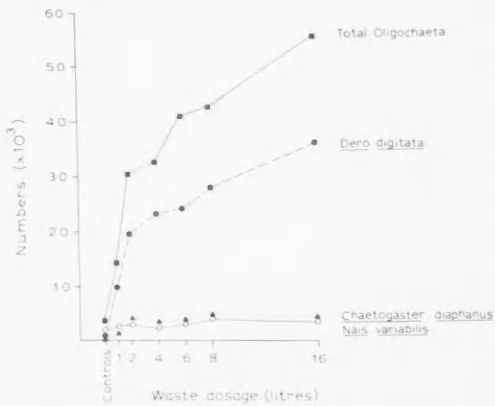

FIG. 13. Response of Oligochaeta to fish feces.

approximately 0.5 m², of riffle substrate 15 days after all the animals (and presumably much of the detrital food material) have been removed. This agrees with the 10–14-day period for reestablishment of populations of *Baetis vagans* McDunnough and *Gammarus limnaeus* Smith in Valley Creek, Minnesota (Waters 1964), and the 8 days observed for Ephemeroptera and Plecoptera to recolonize trays of gravel in a stream in Lapland (Ulfstrand 1968). Although the present study shows a recovery of total numbers after 15 days, curves for some species (e.g. *Baetis* sp. A and *Simulium arcticum*) suggest that recolonization may only just have been completed after 24 days, so the 4-wk period suggested by some workers, e.g. Mason et al. (1967) and Crowe (unpublished data), may be more appropriate in studies where complete recolonization of all the taxa is required (e.g. Williams and Hynes 1976). The full recovery of total numbers noted after only 2 wk could be due to the overcompensation by some species, as for example, in the case of *Nais variabilis* and *Phaenopsectra*. Waters (1964) found that *Baetis* initially recolonized well above its usual density at a time when *Gammarus* was below its own density norm, but that this imbalance was soon corrected. Perhaps these temporal fluctuations reflect the accumulation patterns of different sizes and types of food particles. One might expect, therefore, that on clean gravel detritus feeders would colonize more quickly than periphyton grazers and predators. This is not to say that detritivores will be the first to arrive, as indeed Fig. 1A indicates that 75% of the taxa were already represented on day 3, but rather that they may be numerically dominant. Further complication will arise because certain grazers may be facultative detritivores, and some predatory species are herbivorous in their early instars.

Despite taxonomic variations and adjustments during recolonization, small areas of gravel, under conditions similar to those found in the rearing channel in winter, may be disturbed every 15 days to yield about 80,000 animals/m², to a depth of 15 cm, weighing approximately 0.62 g, dry weight (95% confidence limits 0.32–0.92). Longer intervals should result in the same number of slightly larger animals.

To apply this finding to feeding fish under natural or seminatural conditions, three things must be considered. First, a suitable method of dislodgment must be selected. The gravel in the experimental troughs was disturbed, adequately, with a garden rake, although compressed air or water jets might be more practical on a large scale. Second, a suitable unit area to be disturbed must be chosen. This should not be so large as to affect the recolonization rate through organisms having further to travel from undisturbed areas. The lower limit will be controlled by the number of fish requiring food, so perhaps an area of between 0.5 and 1 m² would be optimal. Third, a dislodging schedule should be prepared taking into account the feeding activity of the fish. For example, it would be better to disturb a single 1 m² area 6 times/day, than one 6-m² area once per day.

Continued cropping of the benthos on a riffle may decrease the amount of natural drift emanating from it (Dimond 1967). However, this loss should be more than compensated for by the animals dislodged artificially. Provided that nearby riffle areas were left untouched to serve as production sites, then optimum food-organism level should be sustained.

The results of Experiment 2 indicate that accumulation of small quantities of O.M.P. (a maximum of 4 ℓ/trough, or 9 ℓ/m^2) increase total numbers and biomass, of benthic standing crop. Increase of important food species (e.g. the Chironomidae) should compensate for the loss of some taxa. It could be argued that even doses as high as 16 ℓ/trough (36 ℓ/m^2) do not cause numbers and biomass to fall below that of clean gravel and so artificial food accumulation should not give cause for concern. However, despite the apparent lack of any change in species diversity between the 4- and 16-ℓ troughs, there was considerable shift in species dominance, i.e. to Orthocladiinae in general and to *Cricotopus/Orthocladius* sp. in particular. Perhaps the danger in promoting such a monoculture lies in the fact that the peculiarities in the life cycle of a single species may make it only periodically available to fish (for example, during its drifting or emerging phases). If we wish to optimize the use of benthos in the diet of young salmonids, a variety of food organisms with asynchronous life cycles should be the objective.

As with Experiment 2, limited quantities of fish feces (i.e. to a maximum of 2 ℓ/trough or 4.5 ℓ/m^2 for numbers and 16 ℓ/trough or 36 ℓ/m^2 for biomass) apparently increase benthos. The degree of enhancement is greater than with O.M.P., but much of this is due to oligochaetes and Chironomini (the latter not being very abundant but large). The same argument against promoting chironomid monocultures, as noted in Experiment 2, can be made here. In general, oligochaetes are not considered important in salmonid diets, because of their inaccessibility, although Aarefjord et al. (1973) have shown their seasonal consumption by trout in Norway. Nevertheless, the fact that this is predominantly a naidid oligochaete fauna rather than a tubificid-dominated one is encouraging as it indicates that bottom conditions, even under a loading of 36 ℓ/m^2, have not degenerated to the stage where oxygen is in short supply and gross organic pollution evident (Brinkhurst and Jamieson 1971).

The question arises as to whether the fauna enhanced by these additives is making use of the material itself, or the microorganisms and aquatic fungi that grow on it. Laboratory culture showed that a thick mat of fungi (primarily the Oomycetes *Saprolegnia* sp., *Phytophthora* sp., and possibly *Achlya* sp.) developed on O.M.P. under 7°C water, flowing at 3 cm/s, in only 7 days. During this period, protozoans, rotifers, and nematodes appeared and were eaten by chironomid larvae which also ate the fungi and O.M.P. Although luxuriant mats of Saproligniaceae may enhance benthos production and may themselves contribute to stream purification by reducing waste organic material, they pose a threat to the fish. Zoospores released by the fungi may settle in open wounds or areas where scales have been lost, and form debilitating, and possibly fatal, growths (Scott 1964).

Fecal pellets, although compact and enclosed in a thick mucous sleeve when fresh, soon become ramified by fungal hyphae and microorganisms. These too provide a food for benthos. Some chironomids, notably *Pentapedilum* and *Phaenopsectra*, construct tubes in fresh pellets and eat the mucous coating. Thus, few toxins exist in the pellets, most of the ammonia, urea, and other metabolic wastes having been expelled through the gills and in the urine. Moreover, Elliott (1976) has shown that the feces of brown trout, *Salmo trutta*, may contain as much as 30% of the energy ingested in its diet.

The occurrence of different dominant species in the high dosage troughs in Experiments 2 and 3 probably reflects a response of benthos to different food microorganisms growing on the substrates.

Clearly then, limited accumulation of fish production wastes is to be encouraged in the type of seminatural rearing channel proposed by Mundie (1974). Fish can benefit directly because the yield of food organisms cropped from small areas of substrate every 15 days will be greater, in some cases 3 times as much. Such increases are similar to those obtained in enrichment experiments using willow leaves, alder leaves, and cereal grain (Mundie et al. 1973). Moreover, in the process of feeding on, and living in, the wastes, the benthos is bringing about a change in its chemical and physical composition. Analogies with sewage purification are apparent (see Lackey 1949).

Acknowledgments

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Effects of Fry and Fingerling Introductions on Walleye (*Stizostedion vitreum vitreum*) Production in West Blue Lake, Manitoba

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SCHWEIGERT, J. F., F. J. WARD, AND J. W. CLAYTON. 1977. Effects of fry and fingerling introductions on walleye (*Stizostedion vitreum vitreum*) production in West Blue Lake, Manitoba. J. Fish. Res. Board Can. 34: 2142–2150.

Walleye (*Stizostedion vitreum vitreum*) were introduced into West Blue Lake, Manitoba, as fry in 1971 and as fry and fingerlings in 1972. Introduced fish were selected to possess malate dehydrogenase (MDH) isozyme phenotypes different from native walleye. The fingerlings were also fin clipped. During the summer months of 1973, 1974, and 1975 fish were recaptured by gill net. Muscle from recaptured fish was used for the determination of MDH isozyme phenotype whereby the contribution of the introduced fish to year-class abundance, biomass, and production was calculated.

The 1971 introduction augmented the year-class by 38% but this brood provided only 1.3% of the total catch during the September 1974 to August 1975 period. In contrast, the 1972 introductions formed 99% of the year-class and contributed 37.8% of the total catch in the same 1974–75 period. The fingerling introduction formed only 7.0% of the 1972 year-class abundance in 1975.

Introduced fish provided 54% of the total walleye production of 260 kg (wet weight) during the period September 15, 1974 to August 8, 1975. Production by introduced fish was apportioned as follows: 1972 fry 91%; 1972 fingerlings 7.4%; 1971 fry 1.7%. This investigation demonstrates the important contribution that introduced walleye fry may make to production and the utility of isozyme "tagging" in the evaluation of fish introductions.

Key words: walleye, *Stizostedion*, production, fry introductions, malate dehydrogenase, MDH, isozymes, fish, introduction evaluation

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SCHWEIGERT, J. F., F. J. WARD, AND J. W. CLAYTON. 1977. Effects of fry and fingerling introductions on walleye (*Stizostedion vitreum vitreum*) production in West Blue Lake, Manitoba. J. Fish. Res. Board Can. 34: 2142–2150.

On a introduit des dorés jaunes (*Stizostedion vitreum vitreum*) dans le lac West Blue, Manitoba, au stade de fretin en 1971 et de fretin et de fingerlings en 1972. Les poissons introduits avaient été sélectionnés selon la possession de phénotypes de l'isozyme de la malate déshydrogénase (MDH) différents des dorés indigènes. On a également marqué les fingerlings par ablation de la nageoire. Au cours des mois d'été 1973, 1974 et 1975, les poissons ont été recapturés à l'aide de filets maillants. Le muscle des poissons recapturés a servi à la détermination du phénotype de l'isozyme de la MDH et on a calculé la contribution des poissons introduits à l'abondance, à la biomasse et à la production de la classe d'âge.

Le peuplement de 1971 augmenta la classe d'âge de 38%, mais cette progéniture ne fournit que 1.3% de la prise totale durant la période de septembre 1974 à août 1975. Par contraste, les peuplements de 1972 constituèrent 99% de la classe d'âge et contribuèrent 37.8% de la prise totale durant la même période de 1974–75. Le peuplement de fingerlings ne constitua que 7.0% de la classe d'âge de 1972 en 1975.

Les peuplements fournirent 54% de la production totale de dorés jaunes de 260 kg (poids humide) durant la période du 15 septembre 1974 au 8 août 1975. La production de poissons introduits se répartit comme suit : fretin 1972, 91%; fingerlings 1972, 7.4%; fretin 1971, 1.7%. Cette étude démontre l'importante contribution que des peuplements de fretin de doré jaune peuvent apporter à la production et l'utilité du « marquage » par isozyme dans l'évaluation des peuplements de poissons.

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ARTIFICIAL propagation and augmentation of depleted fish stocks is a widely practiced and generally accepted management technique, but the efficacy of this approach has seldom been examined. Results obtained from alternate year introductions or those made during a sequence of years (Rose 1955; Forney 1975, 1976; Carlander et al. 1960) have, however, indicated enhancement of some year-classes of the walleye (*Stizostedion vitreum vitreum*), perhaps because high stocking densities were used. This report describes an investigation of the contribution of introduced walleye fry and fingerlings to year-class strength, biomass, and production. Introduced fish were biochemically distinguishable from native fish.

The walleye of western Canada exhibit six phenotypes for the cytoplasmic form of malate dehydrogenase (MDH) which are determined by three alleles at one genetic locus (Clayton et al. 1971). The geographic distribution of these MDH alleles is not uniform among walleye populations, and while alleles c^2 and c^3 predominate in West Blue Lake walleye, the c^1 allele has been found at high frequency in only a few populations (Clayton et al. 1974). On the basis of this knowledge egg lots, and ultimately fry, with only the C^1C^1 and C^1C^3 phenotypes were selected from an appropriate population for introduction into West Blue Lake, where the native fish are substantially only of phenotypes C^2C^2, C^2C^3, and C^3C^3.

The small closed-system environment of West Blue Lake, with an unexploited native walleye population and no major predator species, was suitable for monitoring and assessing the success of fry and fingerling introductions. In addition, considerable background information was available on year-class strengths, growth, seasonal changes in biomass, and production of the native walleye population (Glenn 1969; Kelso and Ward 1972). These data provide a comprehensive standard against which effects of stocking can be assessed.

Initial monitoring indicated that the small introduction of walleye fry in 1971 was moderately successful, while the larger stocking in 1972 apparently created an abundant year-class (Ward and Clayton 1975). The objectives of the present study were to continue to monitor the introductions and to evaluate quantitatively the effects of the introductions on population abundance, biomass, and production.

Materials and Methods

West Blue Lake is located in Duck Mountain Provincial Park, approximately 500 km northwest of Winnipeg, Manitoba (Fig. 1). The lake, 160 ha in area, is long, narrow, and steep-sided with three basins. The maximum length is 4.8 km, maximum depth 31 m, and mean depth 11.3 m. There is no outlet stream and only two small temporary inlet streams which drain marshes to the west (Ward and Robinson 1974).

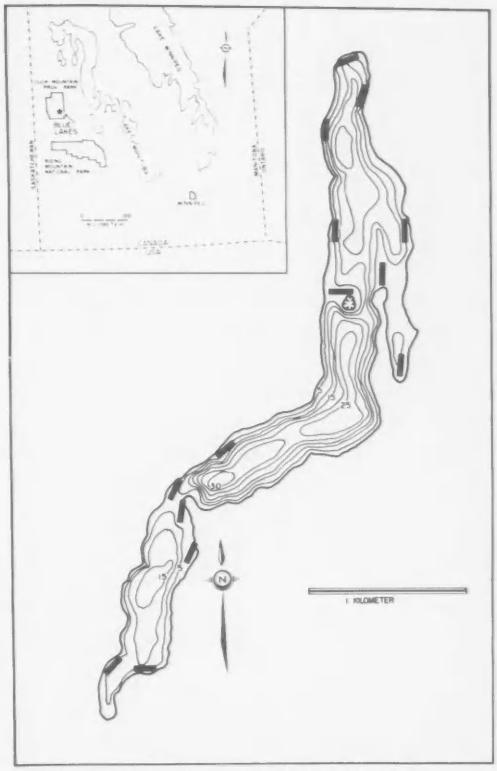

FIG. 1. Bathymetric map of West Blue Lake, contours at 5-m intervals. ■, fishing sites.

The genetics of three alleles at one of the two loci for supernatant MDH in walleye have been described previously, together with methods for the determination of the phenotype of individual fish (Clayton et al. 1971).

The parental walleye stock used to provide fry for the West Blue Lake introductions overwinter in Crean Lake, Prince Albert National Park, Saskatchewan, but spawn in adjacent streams in spring. Prespawning fish were trapped in a tributary of Crean Lake and retained in pens until ripe.

In early May, 1971, 36, 1 × 1 matings were made and fertilized eggs were held at the spawning site. Egg lots representing matings of the type $C^1C^1 \times C^1C^1$ and $C^1C^1 \times C^1C^3$ were selected for the subsequent fry plant into West Blue Lake and were incubated in a small field hatchery in Prince Albert National Park. Later in May the eggs were transferred to the provincial hatchery at Duck Bay, Manitoba and 50,000–100,000 resultant fry were released into West Blue Lake on June 1.

In 1972 the phenotypes of the parental fish were

determined prior to spawning and matings of the type $C^1C^1 \times C^1C^2$ were the only ones made. During the period May 3–8, phenotype selections were made as follows: potential spawners were anesthetized with MS-222®, (*m*-aminobenzoic acid ethyl ester methanesulphonate, Kent Chemicals, Vancouver); then a small sample of muscle was removed from the back of each fish with a modified hypodermic needle (Uthe 1971) and each fish was marked with a numbered tag. The tagged and sampled fish were returned to a holding pen at the spawning site. Isozyme phenotypes were determined within 2 days of sampling at a field laboratory. The C^1C^1 and C^1C^2 fish were then marked with a distinctive colored tag to facilitate selection at spawning time. The desired matings were made during the period May 7–13 using groups of four to six fish. Egg lots from male/female reciprocal matings were kept separate up to and through the water hardening stage to eliminate the possibility of any stray fertilizations.

Egg lots were combined after water hardening at the spawning site and were transported to the Saskatchewan Department of Natural Resources, Fort Qu'Appelle fish hatchery for incubation. The combined egg lots were transferred on May 26 to a small field hatchery constructed on the shore of West Blue Lake. The eggs were treated with an iodine containing organic disinfectant (Wescodyne®) upon arrival at West Blue Lake and were incubated until hatching was completed on June 4. On June 6, groups of fry were transferred from holding tanks to a large, shallow, plastic tray and photographed. Subsequently, the negatives were developed and projected onto a gridded screen, and the fry were counted.

The fry were subdivided into two lots; the largest fraction, 373,000, were released at various locations in West Blue Lake and the remainder, 186,000, were transferred to two small ponds near Erikson, Manitoba. In October 1972, 2500 fingerlings were captured, transported to West Blue Lake, finclipped, and released into the lake.

Sampling procedures for population enumeration during 1972–75 consisted of capturing fish in standard gangs of gill nets composed of three sections each 30.5 m long and 1.8 m deep. Mesh sizes were 3.81, 6.35, and 8.89 cm stretched measure (Kelso and Ward 1972). The nets were set between 2000 and 2200 h at an acute angle to shore and usually in < 5 m of water (Fig. 1). The nets were checked for fish at 20–30-min intervals or more frequently if the catch was heavy. During the 1972–74 period, two gangs were fished each day of sampling but in 1975 two to four gangs were fished. Fish were separated according to the mesh size of capture beginning in August 1974.

Fish were removed from the nets as soon after capture as possible, placed in a 25-ℓ container of water, and then transferred to a "live box," where they were retained overnight (Kelso and Ward 1972). The next morning, fish in good condition were measured (total and fork lengths), and scale samples were taken from the left side above the lateral line between the two dorsal fins. Floy FD-67 individually numbered anchor tags (Dell 1968) were inserted in the back below the middle of the first dorsal fin. During the summer of 1975 two tags were applied, one on each side, to assess tag loss.

In 1973, 10% of the standard gang gillnet catch was killed for MDH analysis. In May 1974 the hypodermic syringe biopsy technique (Uthe 1971) was tested as a means of obtaining tissue samples without killing the fish. Concomitant with the first three mark–recapture periods of 1974, control and biopsied fish were held in 560-ℓ tanks on the lakeshore to assess mortality resulting from capture and handling and the biopsy procedure. As a result of this experiment, in June 1974 the hypodermic syringe method was used exclusively to obtain tissue samples from every second fish in 1974 and every fifth individual from standard gang catches in 1975.

A total of five mark-and-recapture experiments were conducted from September 1974 through 1975. Population abundance estimates were determined for all periods by the Jolly–Seber multiple release procedures (Jolly 1965) modified to adjust for small sample bias (Seber 1973).

Age composition of catches was estimated from age analyses of scales. All scales were read twice, disagreement resulting in further reading. Only scales meeting the criteria of Hile (1954) were used for determining age. Of the 2523 fish captured over the 4 yr 2437 individuals could be used. When determining the brood year, the last week of June was taken as the time of annulus formation (Glenn 1969), unless an annulus was obviously present prior to that period.

Instantaneous rates of growth in weight (G) were determined indirectly from length measurements. All length measurements were converted to weights using the relationship: $\log w = 3.162 \log l - 5.463$, where w is weight in grams and l is total length in millimetres (Glenn 1969). The catches were subdivided into age-classes using age-frequency data obtained from scale readings. A mean weight for each age-class in each catch was then calculated. Instantaneous growth rates were calculated for periods between sampling or catch periods using $G = \ln w_2 - \ln w_1$, where w_1 is the mean weight of a year-class at sampling period 1 and w_2 is the mean weight of the same year-class at sampling period 2.

The biomass of each year-class (1966–73 inclusive) was calculated by multiplying the mean weight of a year-class by the number of individuals in it. Biomass estimates were available for four sampling times during 1974 and 1975. Average biomass ($\bar{B}$) of the stock was the arithmetic mean of adjacent biomass estimates (Chapman 1968) and production was calculated with the Chapman modification of the Ricker (1946) approach, $P = G\bar{B}$, where G is the instantaneous growth in weight.

Production of introduced fish was apportioned on the basis of their frequency in the population as derived from isozyme determinations. The 1972 introduction was subdivided into fish introduced as fingerlings (finclipped fish) and fish introduced as fry, in catches during 1974 and 1975. Production of introduced fish was determined by Chapman's arithmetic procedure.

TABLE 1. Number of walleye with known malate dehydrogenase isozyme phenotype from samples from standard gang gillnet catches, 1973–75.

Year-class	Phenotypes															Totals			Fraction introduced		
	C^1C^1			C^1C^3			C^2C^3			C^2C^2			C^3C^3								
	73	74	75	73	74	75	73	74	75	73	74	75	73	74	75	73	74	75	73	74	75
1973	—	0	0	—	0	0	—	0	1	—	3	4	—	0	2	—	3	7			
1972	—	50	60	—	45	54	—	2	1	—	0	1	—	0	0	—	97	116	0	.98	.98
1971	1	1	2	2	2	1	3	4	2	0	3	2	0	1	1	6	11	8	.50	.27	.38
1970	0	0	0	0	0	0	5	2	1	3	2	0	2	1	0	10	5	1			
1969	0	0	0	0	0	0	2	1	1	1	1	0	0	1	0	3	3	1			
1968	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	2	0			
1967	0	0	0	0	0	1	13	31	14	13	37	13	4	10	0	30	78	18			
1966	0	0	0	0	0	0	1	0	1	0	3	0	1	0	0	2	3	1			
													Totals			51	202	152			

Results

Throughout this investigation two year-classes of walleye (1967 and 1972) predominated (Fig. 2), and in 1975 they contributed 48 and 38%, respectively, to the total catch. Other year-classes contributed little to standard gang catches in 1975 and previously. The data indicate that large year-classes occur infrequently.

In 1973, MDH analyses of samples indicated that the 1971 brood contained 50% introduced fish (Table 1). By 1974 both augmented year-classes were fully vulnerable to sampling and analyses indicated that fish introduced as fry accounted for 27 and 98%, respectively, of the 1971 and 1972 broods. Similar proportions were found in 1975 when the 1971 introduction formed 38% of that year-class present in samples analyzed for isozymes and the 1972 introduction contributed 98% of the 1972 year-class. If it is assumed that the proportions of introduced fish among those sampled for MDH from standard gang catches (Table 1) were representative of those in the population, the combined results of all isozyme analysis suggest that 36% of the 1971 brood (9 from a total of 25) and 98% of the 1972 brood (209 from a total of 213) were introduced fish (Fig. 2). The abundant 1972 year-class constituted 38% of the total catch in 1975. Only four native fish, two each in 1974 and 1975, were identified by MDH analysis. The remainder (98%) were introduced.

Introduced fingerlings contributed only 9% (14 individuals) of the 1972 year-class in 1974 and 7% (24 fish) in 1975 (Fig. 2). These marked fish were not included in those analyzed for isozyme phenotypes (results are shown in Table 1). When the fingerling recaptures are considered in addition to fry contributions, introduced fish supplied the following to the catch of the 1972 brood:

1973	1974	1975
1 (100%)	109 (98.2%)	138 (99%)

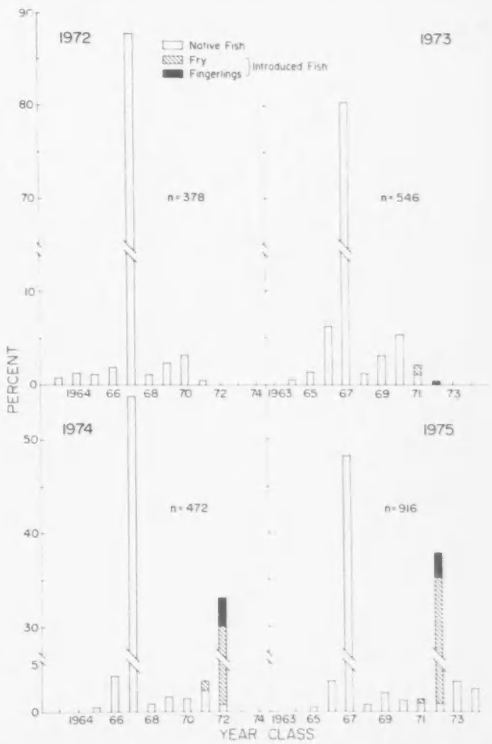

FIG. 2. Relative year-class strengths of West Blue Lake walleye from catches (n) and the contributions of introduced fish.

Abundance ($\hat{N}$) estimates (Table 2) indicated a stable population, generally between 2000 and 3000 individuals. Seasonally, recruitment and mortality interacted to modify population size similar to the pattern described by Kelso and Ward (1972). Survival ($\hat{\phi}$) exceeded 80% during all intervals. Recruitment occurred primarily during the summer period, early June through late August.

Growth in weight of native and introduced fish of the 1971 brood was similar (Fig. 3). Intercepts of both regression lines coincided at a ln value of 4.7 and the instantaneous growth rates were 0.0020 for native fish and 0.0017 for introduced fish. Fingerlings introduced in the fall of 1972 were much larger than fry released previously in June (Fig. 3). Although this weight advantage was maintained, the size differences between the two groups decreased with time, i.e. the instantaneous growth rate for introduced fry ($G = 0.0033$) was higher than the value for introduced fingerlings ($G = 0.0018$). Too few native fish of the 1972 year-class were available for quantitative comparison but their weights at comparable times were intermediate between weights of the two groups of introduced fish (Fig. 3).

Total production, September 15, 1974 to August 8, 1975, for all age-classes between 1966 and 1973 inclusive was 260 kg, of which 92 kg (35%) was contributed by the 1967 year-class and 140 kg (54%) by the 1972 year-class (Table 3). In August 1975 the biomasses of the two dominant year-classes showed a reversal of the production estimates, e.g. the biomass of the fully recruited 1972 year-class was 528 kg, while that of the 1967 brood, 699 kg (Table 3). The reason for high production from the younger brood, in spite of their smaller biomass, is apparent from a comparison of the instantaneous growth rates. The appropriate G-value for the 1967 brood was 0.1001 and for the 1972 year-class, 0.3761 (Table 3).

The production of introduced fish was examined separately for the two augmented year-classes (Table 4). Fish introduced as fry contributed 2.44 kg (38%) of the 1971 year-class production and over 90% of the 1972 brood total production. Fish introduced as fingerlings provided over 7% of the 1972 year-class production. In toto, introduced fish accounted for 140.82 kg or 54.0% of the production for the September 1974 to August 1975 period. Of this total, 90.8% was contributed by fry released in 1972, 7.4% by fingerlings released in the fall of 1972, and 1.7% by fry released in the spring of 1971.

TABLE 2. Population parameters estimated using the birth and death model of Jolly (1965). Standard errors are in parentheses.

Date[a]	Abundance ($\hat{N}$)	Survival ($\hat{\phi}_i$)	Recruitment ($\hat{B}_i$)
Sept. 15, 1974	2774(1687)	.8401(.1832)	−547(1387)
May 18, 1975	1784(482)	.8770(.1915)	473(454)
June 17, 1975	2037(493)	.9594(.3347)	1051(615)
Aug. 8, 1975	2967(1047)		

[a]Refers to midpoint of sampling period.

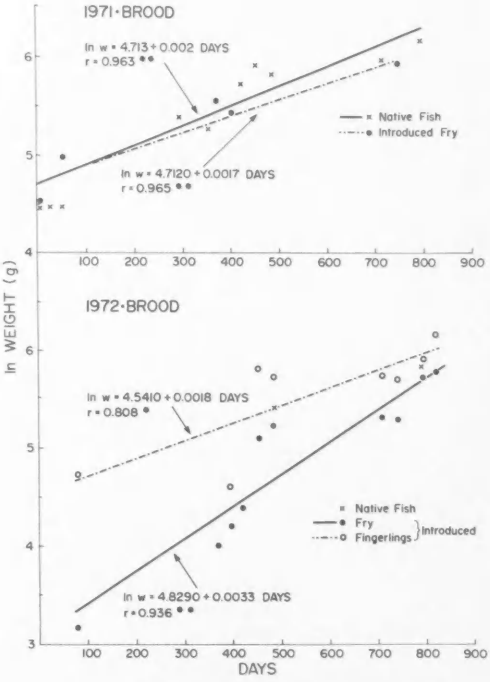

FIG. 3. Growth of native and introduced walleye in West Blue Lake from 1973 to 1975.

Discussion

Present results (Table 1 and Fig. 2) confirm initial indications (Ward and Clayton 1975) that the biologically marked walleye fry introduced into West Blue Lake made significant numerical contributions to their year-classes. The small 1971 fry introduction made up almost half of that weak year-class, while the large 1972 fry introduction produced virtually an entirely artificial year-class rivaling the dominant 1967 brood in abundance. The 1972 fingerling introduction made only a minor contribution to the 1972 brood. The success of the fry introductions is conclusive; furthermore, the constancy of the proportions of introduced fish in the year-classes after the fish reach a catchable size (Table 1) indicates that

TABLE 3. Biomass and production estimates for West Blue Lake walleye year-classes for the period September 15, 1974 to August 8, 1975.

Date	Year-class	Mean wt $\bar{w}$ (g)	Abundance[a] N	Biomass B (kg)		Mean biomass $\bar{B}$(kg)	Instantaneous growth rate G	Production[b] P (kg)	Year-class production (kg)[c]	
Sept. 15	1966	1113	117	130.22						
1974	1967	802	1343	1077.09						
	1968	684	58	39.67						
	1969	576	58	33.41						
	1970	652	31	20.21	1966	136.05	.0116	1.58		
	1971	372	58	21.58	1967	1058.10	−.0125	−13.23		
	1972	206	1021	210.33	1968	22.29	−.1096	−2.44		
	1973	95	89	8.46	1969	36.02	.0623	2.24		
			2775	1540.97	1970	14.96	−.0715	−1.07		
					1971	12.13	−.1078	−1.31		
May 18	1966	1126	126	141.88	1972	131.40	.0428	5.62		
1975	1967	792	1312	1030.10	1973	4.23	—	—		
	1968	613	8	4.90		1415.18		−8.61		
	1969	613	63	38.62						
	1970	607	16	9.71	1966	102.45	.0357	3.66		
	1971	334	8	2.67	1967	932.55	.0311	29.00		
	1972	215	244	52.46	1968	8.17	.2176	1.78		
	1973	—	—	—	1969	30.62	−.0553	−1.69		
			1777	1289.34	1970	13.46	−.0896	−1.21		
					1971	10.17	.1395	1.42		
June 17	1966	1167	54	63.02	1972	113.29	.0184	2.08		
1975	1967	817	1011	825.99	1973	2.12	—	—		
	1968	762	15	11.43		1212.83		35.04		
	1969	580	39	22.62						
	1970	555	31	17.21	1966	63.02	.0187	1.18		
	1971	384	46	17.66	1967	762.46	.1001	76.32		
	1972	219	795	174.11	1968	16.29	.0272	.44		
	1973	92	46	4.23	1969	19.22	.0103	.20		
			2037	1136.27	1970	17.90	.2148	3.84	1966	6.42(2.5)
					1971	19.23	.3279	6.31	1967	92.09(35.3)
Aug. 8	1966	1189	53	63.02	1972	351.19	.3761	132.08	1968	.22 —
1975	1967	903	774	698.92	1973	17.74	.7715	13.69	1969	.75(0.3)
	1968	783	27	21.14		1267.05		234.06	1970	1.56(0.6)
	1969	586	27	15.82					1971	6.42(2.5)
	1970	688	27	18.58					1972	139.78(53.6)
	1971	533	39	20.79					1973	13.69(5.2)
	1972	319	1656	528.26						260.49
	1973	199	157	31.24						
			2760	1397.77			Total	260.49		

[a]Abundance estimates do not include members of year classes prior to 1966 or subsequent to 1973; therefore totals do not always coincide with values in Table 2.

[b]$P = G\bar{B}$.

[c]Figures in parentheses are year-class production estimates expressed as percentages of the total.

TABLE 4. Production from components of the 1971 and 1972 broods.

Brood	Total production (kg)	Proportion introduced		Production (kg)		
		Fry	Fingerlings	Fry	Fingerlings	Native
1971	6.42	0.380	—	2.44	—	3.98
1972	139.78	0.915	0.075	127.90	10.48	1.40

mortality rates of native and introduced fish were similar.

Accuracy of production estimates depends on the precision of population estimates (Chapman 1967). Sources of error applying to the West Blue Lake situation were evaluated by Kelso and Ward (1972). The importance of gillnet selection as a source of nonrandom or unrepresentative samples is unresolved (Hamley 1975). Kelso and Ward (1972) found that while meshes were size selective, all sizes of fish were represented in the catch. Temporal changes occurred in the size frequency of catches in the three mesh sizes but these changes resulted from growth rather than selection.

Abundance estimates (Table 2) indicate a pattern similar to that reported by Kelso and Ward (1972). Seasonal changes are also similar with mortality largely restricted to the overwinter and spring postspawning periods. Both sets of estimates were subject to continuous recruitment by 1+ and 2+ individuals during the summer. Survival rates, $\hat{\phi}$ (Table 2) were generally higher, particularly over the winter, than those reported previously (Kelso and Ward 1972). Recruitment was estimated to be 1524 for 1975 (Table 2), similar to the value reported for a comparable segment of the year by Kelso and Ward (1972).

The age composition of the unexploited West Blue Lake walleye population is considerably different from that described for unexploited northern salmonid populations (Johnson 1972, 1973). Only two year-classes were abundant, one group of older fish (1967 brood) and one group of newly recruited fish (1972 brood). These two groups contributed 86% of the 1975 catch. Glenn (1969) also found that the 1963 (3+) and 1964 (4+) year-classes predominated in 1966–67, while Kelso and Ward (1972) found the 1967 (2+) and 1964 (5+) year-classes to be abundant in 1969–70. Age distribution has been unstable for over a decade and weaker year-classes are the rule with an occasional abundant year-class. Although the strong 1972 year-class is composed principally of introduced fish, no depressing effect of the introduction was evident because the 1973 and 1974 native year-classes were abundant.

Production during the period September 1974 to August 1975 was 260 kg (0.32 $g \cdot m^{-2}$) (Table 3). Kelso and Ward (1972) estimated production for a slightly longer period to be 340 kg (0.42 $g \cdot m^{-2}$). Production for approximately comparable periods, May–September in 1969–70 and May–August 1975, was 307 kg (0.38 $g \cdot m^{-2}$) and 269 kg (0.33 $g \cdot m^{-2}$), respectively. Apparently production during the earlier study was slightly higher. Comparable production figures in the literature are scarce but walleye production is low relative to values attributed to salmonid populations (Chapman 1967). Production of West Blue Lake walleye was similar to the 0.13–0.26 $g \cdot m^{-2} \cdot yr^{-1}$ estimated for the Dexter Lake walleye population in northwestern Ontario (Moenig 1975) and to the values of 0.33–0.76 $g \cdot m^{-2} \cdot yr^{-1}$ estimated for Oneida Lake, New York, by Hoffman (1972). Johnson (1966) reported production of from 0.14 to 0.51 $g \cdot m^{-2} \cdot yr^{-1}$ for northern pike (*Esox lucius*) in Lake Windermere.

These comparisons indicate that West Blue Lake walleye production is similar to walleye production in other temperate lakes, although the morphometry of West Blue Lake is not typical (Regier et al. 1969). A basis for future comparisons is provided by these results.

During the September 1974–August 1975 period introduced fish were 3+ and 4+ yr old and contributed the majority (54%) of the total production, indicating that fish introductions may significantly augment production by natural populations. In fact, without the introductions, production would have been only 120 kg (0.15 $g \cdot m^{-2} \cdot yr^{-1}$), although reduced competition would have enhanced growth rate, and therefore, production. The production by introduced fish resulted almost entirely (91%) from the 1972 fry plant, with only 7.4% attributable to fingerlings.

Success of fry introductions is apparently related to stocking density. Early studies (Hile 1937; Carlander 1945; Smith and Krefting 1954), employing low stocking densities (0.004–0.030 $fry \cdot m^{-2}$), showed minimal effects on year-class strength, while higher densities (0.45–2.35 $fry \cdot m^{-2}$) indicated apparent enhancement of year-classes (Rose 1955; Carlander et al. 1960; Forney 1975, 1976). Similarly, the 1971 fry introductions at West Blue Lake (0.06–0.12 $fry \cdot m^{-2}$) met with limited success, while the 0.457 $fry \cdot m^{-2}$ planted in 1972 produced excellent results.

The introduction of 2500 large fingerlings at the end of their first growing season did not make a major contribution to either biomass or production. Similar results have been obtained previously. Olson and Wesloh (1962) were unable to detect increases in gillnet catches resulting from unmarked fingerling introductions. Schneider (1969) reported $< 5\%$ returns of marked fingerlings, while Kempinger and Churchill (1972) and Jennings (1970) obtained returns of 13 and 10% from successful fingerling introductions. Other results were uniformly low, of the order of 1–2%. Survival rates of fingerlings planted in West Blue Lake from release to 1975 were also

about 1%. The fingerling plant in West Blue Lake in 1972 (Table 4) also met with marginal success in terms of production; only 6 kg or 2.3% of September 1974 to August 1975 production was contributed by these fish. The results of our fingerling introduction indicate that additional research needs to be conducted before the efficacy of fingerling introductions can be adequately evaluated.

Comparison of growth rates and intercepts (Fig. 3) for native and introduced fish of the 1971 year-class indicate that differences in size were minor throughout the investigation. The virtual absence of native fish makes the same comparison for the 1972 brood difficult, but the scanty available growth data (Fig. 3) do indicate a similarity between native fish and fish introduced as fry. Although the fish introduced as fingerlings in 1972 were initially much larger than introduced fry of the same age, this initial size advantage was largely lost by late summer 1975 (Fig. 3). Apparently, the only potential long-term advantage in introducing walleye as fingerlings is that the assumed higher survival rate does, in fact, apply.

Explanations for the apparent superiority of fry as opposed to fingerling plants are not readily apparent. It is almost axiomatic that recruitment is a function of stock biomass (Ricker 1954, 1975; Beverton 1962; Beverton and Holt 1957), although Bagenal (1973) demonstrated that fecundity varies to regulate population abundance, with small populations producing more eggs per unit stock than large ones. Initially, large egg depositions experience very intense mortality shortly after spawning. Year-class strengths appear to be determined by the end of the 1st yr. One might therefore expect fingerling introductions to be relatively more successful than fry introductions.

Perhaps fry introductions will be most successful in populations in which natural reproduction and survival rates are usually low or the rate of exploitation of adults is high. Consequently, depensatory predation (negatively density dependent) on young walleye (Forney 1976) is not significant. Apparently the walleye population in West Blue Lake is limited primarily by poor natural reproduction. In this case stocking can produce significant results if food sources are abundant.

The use of biochemically distinctive fry for introductions has been highly successful in providing a means to assess the contribution of the introduced fry to abundance and production. This contribution of introduced fry has been shown to be considerable. Apparently this study represents the first conclusive demonstration that the introduction of newly hatched walleye fry can make an important contribution to an established walleye population. Other introductions with distinctive fry could be envisaged based upon the distribution of MDH alleles in walleye populations presented by Clayton et al. (1974).

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Oocyte Maturation and Ovulation Induced in Hypophysectomized Winter Flounder (*Pseudopleuronectes americanus*) by Preparations from Pituitary Glands of American Plaice (*Hippoglossoides platessoides*)[1]

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CAMPBELL, C. M., AND D. R. IDLER. 1977. Oocyte maturation and ovulation induced in hypophysectomized winter flounder (*Pseudopleuronectes americanus*) by preparations from pituitary glands of American plaice (*Hippoglossoides platessoides*). J. Fish. Res. Board Can. 34: 2151–2155.

Hypophysectomized winter flounder (*Pseudopleuronectes americanus*) did not undergo spontaneous oocyte maturation, but the glycoprotein fraction of extracts of American plaice (*Hippoglossoides platessoides*) pituitaries induced maturation and ovulation. The nonglycoprotein fractions, previously shown to contain a vitellogenic factor, were less efficient than the glycoprotein fractions in the induction of maturation and ovulation. The activity of the nonglycoprotein fraction was further reduced by rechromatography on Con-A Sepharose, suggesting that its action on oocyte maturation and ovulation was due to contamination by the glycoprotein gonadotropin.

Key words: hypophysectomy, gonadotropin, oocyte, maturation, ovulation, vitellogenesis, flounder, plaice, *Pseudopleuronectes*, *Hippoglossoides*

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La plie rouge (*Pseudopleuronectes americanus*) hypophysectomisée ne présente pas de maturation ovocytaire spontanée, cependant les fractions glycoprotéiques obtenues à partir d'hypophyses de la plie canadienne (*Hippoglossoides platessoides*) induisent la maturation et l'ovulation. Les fractions non-glycoprotéiques, dont la présence d'un facteur vitellogénique a déjà été signalée, sont moins efficaces dans l'induction la maturation et l'ovulation. L'activité de la fraction non-glycoprotéique est encore diminuée après un deuxième passage sur une colonne de Con-A-Sépharose suggérant que l'activité sur l'ovulation est due à une contamination par la gonadotropine glycoprotéique.

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IN recent years there has been a growing interest in the purification of teleostean glycoprotein gonadotropic factors (salmon: Donaldson et al. 1972; Idler et al. 1975a; Pierce et al. 1976; Yoneda and Yamazaki 1976; carp: Burzawa-Gerard 1971; Sinha 1971; sturgeon: Burzawa-Gerard et al. 1976). These preparations have been shown to have activity in various teleostean bioassay systems. The stimulation of adenyl cyclase activity in gonads in vitro (Fontaine et al. 1970; Idler et al. 1975b) and the induction of oocyte maturation and ovulation in vivo (Yamazaki and Donaldson 1968; Sinha 1971; Ishida et al. 1972; Sundararaj and Anand 1972; Shehadeh et al. 1973) and in vitro (Jalabert et al. 1972; Hirose and Donaldson 1972; Sundararaj et al. 1972a) are assays involving either an unknown physiological function or a function terminating gametogenesis.

The action on vitellogenesis of pure hormone preparations has not been adequately evaluated. In an earlier study of vitellogenesis Campbell and Idler (1976) found that the nonglycoprotein fraction of an extract of *Hippoglossoides platessoides* pituitaries induced yolk incorporation in the ovary. (Nonglycoprotein refers to the protein not bound to Con-A Sepharose and therefore is

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TABLE 1. Fractions of *H. platessoides* pituitary extract

	Unbound to Con-A Sepharose (nonglycoprotein)	Bound to Con-A Sepharose (glycoprotein)
Extract from 28-g batch A (as used by Campbell & Idler 1976)	Con A_1a	Con A_2a
Total protein (mg)	1302	41
Extract from 18-g batch B (May 1975)	Con A_1b	Con A_2b
Total protein (mg)	1110	135
Rechromatography of unbound fraction Con A_1b	Con A_1b_1	Con A_2b_2
Total protein (mg)	290	1.4

more accurately described as not having α-D-mannopyranosyl or α-D-glucopyranosyl residues.) Since the glycoprotein fraction (which would contain the classical gonadotropins of the types previously isolated) failed to stimulate vitellogenesis, it is important to evaluate which of these fractions was capable of inducing oocyte maturation (biochemical and morphological changes associated with resumption of meiosis) and ovulation. Previous results (Campbell 1975) had shown *Pseudopleuronectes americanus* to be a good test animal, since hypophysectomy abolished spontaneous oocyte maturation and ovulation which resumed only after treatment with gonadotropins.

Materials and Methods

Animals and Evaluation of Ovarian Stage

Fish were collected by divers during the period of normal spawning (May–July), maintained in tanks of running seawater (4–6°C), and hypophysectomized as described by Campbell and Idler (1976). No fish matured more than three days after hypophysectomy and most hypophysectomized fish did not mature; therefore, fish no longer maturing due to hypophysectomy were selected for injection 3 days after surgery.

Biopsies of 20–100 oocytes were taken daily with a Silverman biopsy needle (14 ga.) inserted via the genital pore. Fish were classified according to the stage of oocyte development: stage 1 — oocytes with central nuclei (initial state in all experiments); stage 2 — oocytes with nuclei partially migrated to the periphery; stage 3 — oocytes with germinal vesicle migration completed; stage 4 — oocytes mature, germinal vesicle breakdown; stage 5 — ovulation.

Pituitary Preparations

Pituitary glands were collected from *H. platessoides* from 2 to 9 days post-mortem, at the Bonavista Cold Storage Fish Plant, Fermeuse, Newfoundland. They were extracted and fractionated using Con-A Sepharose (Pharmacia Fine Chemicals) under the conditions previously described (Idler et al. 1975a; Campbell and Idler 1976). For a description of the samples used in the present study see Table 1.

Experimental Protocols and Results

Effect of Hypophysectomy and Response to Injection of Glycoprotein (Con A_2)

To determine the effect of hypophysectomy and response to a single injection of a glycoprotein isolated from the pituitary extract, six fish were given 13 mg Con A_2a/kg body wt and five were injected with saline on the 3rd day post-hypophysectomy. The fish were checked daily for 10 days.

No control fish showed any sign of oocyte maturation during 10 days of observation. One half of the animals treated with Con A_2a had oocytes with migrated germinal vesicles on the 4th day after injection. One fish died before maturing but all other glycoprotein-treated fish were mature (two fish) or had ovulated (three fish) on the 10th day.

Comparison of Activity of Glycoprotein (Con A_2) and Nonglycoprotein (Con A_1) Fractions

To compare glycoprotein and nonglycoprotein fractions, six fish were treated with 0.7 mg Con A_2a/kg and six others with 25 mg Con A_1a/kg on the 3rd, 5th, and 7th day after hypophysectomy; they were checked daily for 12 days.

A simplified summary of the results, presented in Fig. 1 by giving a value to each oocyte stage (stage 1 = 0; stage 2 = 5; stage 3 = 10; stage 4 = 15; stage 5 = 20) and calculating a daily score for each group, shows that Con A_2a induced germinal vesicle migration in three fish before the third injection, whereas only one Con A_1a-treated fish showed a similar response. On the

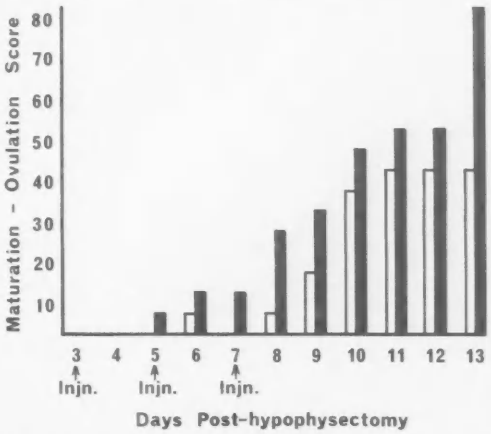

FIG. 1. Daily maturation–ovulation score for groups of fish treated with glycoprotein fraction (■) or nonglycoprotein fraction (▭) of *H. platessoides* pituitaries.

10th day of observation, all glycoprotein but only three nonglycoprotein-treated fish were mature or had ovulated.

COMPARISON OF RESPONSES TO THREE INJECTIONS OF NONGLYCOPROTEIN BEFORE (Con A_1b) AND AFTER (Con A_1b_1) RECHROMATOGRAPHY ON Con-A SEPHAROSE

To determine the response to three injections of the nonglycoprotein fraction before and after rechromatography on Con-A Sepharose, the fish that served as controls in the first experiment, together with one freshly hypophysectomized fish, were divided into two groups of three fish each 16 days after hypophysectomy and injected with 33 mg/kg of Con A_1b or Con A_1b_1. The injections were repeated twice more on alternate days and the fish observed for 11 days. Thirty days after hypophysectomy two fish, which had not matured, were treated with 4.8 mg Con A_2b/kg (three injections on alternate days) to prove that these fish were capable of a response to gonadotropin.

At the time of the third injection one fish from each treatment had begun maturation and in addition the oocytes of the two other Con A_1b-treated animals had begun germinal vesicle migration. On the 8th day of observation all fish treated with Con A_1b and one fish treated with Con A_1b_1 had ovulated but two Con A_1b_1-treated animals showed no signs of maturation even after 15 days of observation. These two fish matured in response to two injections of the glycoprotein Con A_2b.

COMPARISON OF RESPONSES TO Con A_1 AND Con A_2 FRACTIONS BEFORE AND AFTER RECHROMATOGRAPHY ON Con-A SEPHAROSE

To compare the response to two injections of the nonglycoprotein fractions before and after rechromatography, three fish were injected with Con A_1b (38 mg/kg) on the 3rd and 5th day post-hypophysectomy and four others received an equivalent treatment with Con A_1b_1. Eighteen days post-hypophysectomy, four fish, which had not matured, were given two injections of 4.8 mg/kg Con A_2b and two others were treated with two to four injections of 0.3 mg Con A_2b_2/kg.

After 9 days of observation one Con-A_1b-treated fish had a small proportion (5%) of mature oocytes but even after 26 days this maturation was not complete, and the ovary contained many degenerating oocytes. Oocytes of the two other Con A_1b and three out of four Con-A_1b_1-treated fish began germinal vesicle migration, while the other Con-A_1b_1-treated animal was unchanged on the 11th day of observation.

In the second part of this experiment one fish treated with Con A_2b as a check on its ability to mature died before maturing but three others responded after two injections. One Con-A_2b_2-injected fish died after two injections, and the other showed no response after four treatments with this low dose.

Discussion

Mammalian gonadotropins and pituitary extracts have been used for many years to induce spawning in teleosts (Pickford and Atz 1957). In addition, hypophysectomy has been shown to block spontaneous oocyte maturation and ovulation which could be initiated by mammalian pituitary or placental gonadotropins (Yamazaki 1965; Sundararaj and Goswami 1966).

We found that hypophysectomy of *P. americanus* blocked maturation and ovulation and that partially purified extracts of pituitaries from another flat fish successfully initiated this process. The glycoprotein fraction (Con A_2) of *H. platessoides* pituitaries was much more effective than the nonglycoprotein fraction (Con A_1) in inducing maturation and ovulation in *P. americanus* when tested at doses equivalent to equal weights of fresh pituitary glands. This suggests that as in other teleosts (carp: Burzawa-Gerard and Fontaine 1972; salmon: Idler et al. 1975a; Pierce et al. 1976) there is a glycoprotein gonadotropin in these pituitaries.

The weak action by the nonglycoprotein seems to have been due to contamination by the glycoprotein, since after rechromatography the non-

glycoprotein fraction was even less active when compared at approximately equal equivalents of pituitary gland. Idler et al. (1975a) found that a single passage on Con-A Sepharose did not remove all of the glycoprotein gonadotropin; the amount of salmon pituitary gonadotropin detected by cyclic AMP and radioimmunoassays in the nonglycoprotein fraction varied from 1–17% of the total (Idler unpublished data). Further evidence of contamination of the nonglycoprotein fraction is that a glycoprotein capable of inducing in vitro maturation of *Salmo gairdneri* oocytes (Jalabert et al. 1974) was isolated from a nonglycoprotein fraction of *Oncorhynchus tschawytscha* pituitaries (Campbell unpublished data). The reduction of activity in Con A_1 after a second chromatography is likely due to removal of contaminant glycoprotein. However, in this study insufficient glycoprotein was recovered from the original nonglycoprotein fraction to permit further testing. By giving smaller doses of Con A_1 the risk of minor contamination with Con A_2 should be eliminated.

Crude salmon gonadotropin, SG G100, stimulated vitellogenic activity in *Heteropneustes fossilis* (Sundararaj et al. 1972a) and had a weak effect in *O. gorbuscha* (Funk and Donaldson 1972). Sundararaj et al. (1972b) stimulated vitellogenesis in *H. fossilis* with a crude fraction of pituitary extracts from *Puntius gonionotus*. Fontaine et al. (1976) used a partially purified gonadotropin from pituitaries of *Cyprinus carpio* to stimulate gonadal growth and vitellogenesis in *Anguilla anguilla*. Burzawa-Gerard (1974), using the purer carp gonadotropin, obtained results which led her to conclude that a single purified protein was capable of supporting all phases of gametogenesis. It is our opinion that the above mentioned hormones were prepared by procedures which would not assure the exclusion of nonglycoprotein hormone. Evidence is presented that separation is difficult even using Con-A Sepharose.

We present here some physiological evidence to support an hypothesis of two pituitary gonadotropic factors which contrasts with the conclusion reached by Bruzawa-Gerard and Fontaine (1972) and Burzawa-Gerard (1974), who worked with highly purified carp gonadotropin. The glycoprotein salmon gonadotropins (Idler et al. 1975a; Pierce et al. 1976) have yet to be fully tested for an action in vitellogenesis. Recently Olivereau (1976) developed arguments for a salmon gonadotropin that is produced by gonadotrops that do not exhibit glycoprotein-staining properties.

In summary, evidence is presented that there is a glycoprotein component in *H. platessoides* pituitaries that induces oocyte maturation and ovulation; a nonglycoprotein is involved in vitellogenesis (Campbell and Idler 1976) and this hormone has limited activity in maturation and ovulation most likely because of contamination by glycoprotein.

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Influence of Ice Cover and Man on the Odd-Chain Hydrocarbons and Fatty Acids in the Waters of Jeddore Harbour, Nova Scotia

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Water samples from Jeddore Harbour, Nova Scotia, that passed through a 400-μm screen were examined at monthly intervals between November 1970 and December 1971. They were found to contain 16–390 μg extractable lipid/ℓ water. The fatty acid content of the lipids was characterized by low levels of unsaturation and a predominance of components of moderate chain length (C_{14}–C_{18}). The content of *n*-alkanes recovered from the water samples varied normally from 1 to 10 μg/ℓ water and could not be given a specific origin, although certain details pointed to local pollution as the primary origin. In particular, pristane and phytane virtually disappeared during the period of winter ice-cover when local boat operations ceased. Neither hydrocarbon nor fatty acid details could be conclusively related to unusually high levels of odd-chain fatty acids found in Jeddore Harbour rainbow smelt, *Osmerus mordax*. The impact of hurricane Beth was unexpectedly low, implying that the unusual freshwater runoff did not mix with the normal salt water or disturb the basically marine character of Jeddore Harbour.

Key words: Jeddore Harbour, odd-chain hydrocarbons, odd-chain fatty acids, dissolved hydrocarbons, pristane, phytane, ice cover, hurricane runoff

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Nous avons examiné à intervalles mensuels entre novembre 1970 et décembre 1971 des échantillons d'eau provenant de Jeddore Harbour, Nouvelle-Ecosse, et ayant passé à travers un tamis de 400 microns. On a constaté qu'elle contenait 16–390 μg de lipides extractibles/ℓ d'eau. La teneur en acides gras des lipides est caractérisée par de bas niveaux d'insaturation et une prédominance de composantes de longueur de chaîne modérée (C_{14}–C_{18}. La quantité de *n*-alcanes récupérée des échantillons d'eau varie normalement de 1 à 10 μg/ℓ d'eau, et on n'a pu leur attribuer d'origine spécifique, bien que certains détails laissent supposer que la pollution locale en soit la cause primaire. Le pristane et le phytane en particulier disparaissent presque entièrement en hiver, alors que l'eau est recouverte de glace et que les bateaux cessent leurs opérations. Nous n'avons pu établir de relation définitive entre les détails des hydrocarbures ou des acides gras et les niveaux exceptionnellement élevés d'acides gras à chaîne impaire observés chez l'éperlan arc-en-ciel, *Osmerus mordax*, de Jeddore Harbour. L'impact de l'ouragan Beth s'est fait moins sentir qu'on s'y attendait, c'est-à-dire que le reuissellement d'eau douce inusité ne s'est pas mélangé à l'eau de mer normale et n'a pas dérangé le caractère fondamentalement marin de Jeddore Harbour.

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JEDDORE Harbour is an almost landlocked body of water, some 20 km^2 in extent, approximately 60 km east of Halifax, Nova Scotia. It is subject, through a narrow channel, to vigorous tidal flushing, which is usually adequate to maintain salinity at levels near that for contiguous open ocean, for example 26.1–29.5‰ in July of 1970 (Baxter 1970). For approximately 4 mo of the year Jeddore Harbour has a stable ice cover.

Jeddore Harbour receives drainage water from a watershed of 110 km^2, an area that includes few private dwellings and no important commercial enterprises. Drainage water may thus be expected to contain lipids of natural rather than abiogenic origin (Ruebelt 1970). The harbor itself is bounded by a number of small population centers. Private households as well as several small-scale fish-cleaning operations are known to contribute to a septic input into the harbor (Baxter 1970). The types of lipids that are commonly found in wastewater effluents have been

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described and are dominated in order by C_{18}, C_{16}, and C_{14} chain lengths (Farrington and Quinn 1972). A smaller, though similar, input of lipids seems possible in Jeddore Harbour. Small fuel oil and diesel oil spills and highway drainage, supplemented by offshore pollutants (Keizer et al. 1977), would be expected to account for most of the foreign hydrocarbon materials forseeable in Jeddore Harbour water. Prevailing winds would, however, carry hydrocarbon contaminants from the Halifax–Dartmouth urban complex, including those from two oil refineries and two oil-fueled thermoelectric power stations.

A very significant biotic contribution to water lipid content may be anticipated. Jeddore Harbour contains many common seaweeds and in addition has large shallow areas covered with sea grasses, primarily *Zostera marina*, known locally as eel grass. The biotic contribution of *Zostera* lipids, including high proportions of odd-chain hydrocarbons (OCHC) (Nishimoto 1974), will thus vary with seasonal cycles as reported for *Spartina alterniflora* by Schultz and Quinn (1973) and Gosselink and Kirby (1974). It is necessary to include microbial fatty acids from growth on detritus generated from decaying grasses (Fenchel 1970). The bottom consists in many areas of a fine ooze with heavy populations of molluscs, arthropods, worms, and other invertebrates which could lead to some turnover of bottom deposits and to organic materials of recent origin being recycled, perhaps in microbially modified form, back into the water column.

Unusually high levels of odd-chain fatty acids (OCFA) have been found in the rainbow smelt, *Osmerus mordax*, from Jeddore Harbour and not in smelt fished elsewhere in the Atlantic provinces (Paradis and Ackman 1976a, b). The smelt collect in this harbor from November onwards to the May–June spawning period. A dietary origin for the smelt OCFA was suspected but no food material containing such acids was known in 1970. Among the potential progenitors of the smelt fatty acids would be OCFA of microbial–detritus origin (Johnson and Calder 1973; Schultz and Quinn 1973) or odd-chain hydrocarbons (OCHC) of algal origin (Winters et al. 1969; Blumer et al. 1971; Youngblood et al. 1971; Nishimoto 1974).

Sampling of water from Jeddore Harbour was therefore initiated in November 1970 in the expectation of detecting some unusual feature of the lipid composition of seawater that might be related to the OCFA in the smelt. It was the intent of the program to examine all waterborne lipids, whether of biotic or abiotic origin, which could be expected to enter the food web at a primary level from whence they would be concentrated, modified if necessary, and assimilated by the smelt. Consequently there was no discrimination between dissolved lipids and those from fine particulate matter.

Experimental

All samples were obtained from tidal water flowing beneath the No. 7 highway bridge where the Salmon River enters the eastern arm of Jeddore Harbour (Baxter 1970). Samples were collected every 4–6 wk and usually on flood tides. A tinned-steel container (20 ℓ) with a fine stainless steel screen (400 μm) insert in the spout (14 cm diam) was dropped from the center at the bridge through the top 2–3 m of water and pulled back up by rope. The water was decanted through the mesh screen into two solvent-cleaned glass containers (40 ℓ capacity) each containing freshly distilled chloroform (750 ml) and a Teflon-covered magnetic stirring bar. Concentrated reagent grade sulphuric acid (5 ml) was added and extraction was carried out at 5°C in the covered containers by stirring briskly for 24 h. The chloroform extracts were pooled and washed twice with distilled water, dried, and then evaporated to dryness under vacuum and weighed. A small fraction of the total lipids was reserved for qualitative thin-layer chromatography (TLC; silica gel G developed in hexane:diethyl ether:acetic acid::90:10:1). The balance of the recovered lipid was saponified by AOCS Method Ca-6b-53 (1973). The recovered fatty acids were converted to methyl esters with 7% BF_3-methanol solution. The nonsaponifiable fraction was used for hydrocarbon analysis.

Methyl esters and hydrocarbons were analyzed by gas–liquid chromatography (GLC) on a Perkin-Elmer Model 900 machine as previously described (Ackman and Hooper 1970; Paradis and Ackman 1975). All methyl ester and hydrocarbon samples were hydrogenated in ethanol with platinum catalyst to simplify identification and quantitation and all results tabulated herein refer to hydrogenated samples.

Hydrocarbons were quantitated by the use of an added internal standard, methyl heptadecyl ketone, added to the seawater at 50 $\mu g/\ell$ before extraction with chloroform. The results were corrected by gas-chromatographic response and extraction-recovery factors based on experiments with mixtures of known hydrocarbons added to distilled water shown to be free of organic material by control analyses. Quantitation of total fatty acids recovered was based on weight recovery of methyl esters when such determinations were possible. Owing to the very small lipid recovery in the December 1970 and January 1971 samples, the recovery of fatty acids has been assumed to be 60% of the determined weight of nonhydrocarbon lipid present in the seawater. These fatty acid weights may thus be somewhat underestimated, relative to hydrocarbons, but all fatty acid figures are minimal, since they have not been augmented by a known extraction recovery factor. After study of fatty acid details extraction tests were carried out

with anteiso C_{19} acid, which is recommended as a suitable internal standard at 100 $\mu g/\ell$ water.

Results and Discussion

Chloroform was selected as a compromise solvent for extraction, as it is basically suitable as a solvent for a wide variety of neutral and polar lipids, including fatty acids (Treguer et al. 1972). All halogenated solvents are not uniformly suitable for extraction of hydrocarbons from seawater (Keizer and Gordon 1973), but in preliminary evaluations of the method chloroform was shown not to discriminate among the C_{12}–C_{20} hydrocarbons or C_{14}–C_{18} fatty acids, a satisfactory consideration in assessing the impact of C_{15} and C_{17} materials anticipated as prime targets for this study. The extraction efficiency for hydrocarbons was shown to be greater than 90%, or comparable to the findings of Iliffe and Calder (1974). The recovery of free fatty acids was regarded as the most difficult case for the lipid classes anticipated, but preliminary experiments with large volumes of distilled water and C_{14}–C_{18} fatty acids showed recoveries into chloroform to exceed 80%.

The lipids from all water samples that were examined by TLC contained polar lipids, pigments, free fatty acids, triglycerides, and hydrocarbons in varying proportions. These chromatographic studies revealed that triglycerides and free fatty acids predominated in total lipids. It is therefore not surprising that weight recoveries of fatty acids were 75% or more for the February 1971 to December 1971 samples, relative to total lipid less unsaponifiable matter, except for May, where this recovery was 57%. The relative amount of hydrocarbons varied greatly, while polar lipids were apparently minor in proportion in most samples as judged by band size and intensity. Trace amounts of other lipids such as mono- and diglycerides, alcohols, sterols, and wax esters were also observed in some samples.

The values obtained for lipid and hydrocarbon levels in Table 1 are of the same order of magnitude as many previously reported for seawater. Comparison with literature results is difficult, especially as our filtration was intended to remove only multicellular plant and animal life. Stauffer and MacIntyre (1970), on the coastal shelf, found most samples to contain from 10 to 25 $\mu g/\ell$ of dissolved fatty acids. Presumably their total lipid levels would be comparable to the range of 16–390 $\mu g/\ell$ found in Jeddore Harbour. Quite similar levels of dissolved fatty acids were found by Treguer et al. (1971, 1972), and Jeffrey (1968) in another study.

Levels of *n*-alkane in Jeddore Harbour, ranging from 1 to 10 $\mu g/\ell$, are lower than many results reported (Barbier et al. 1973; Pastorelli and Chiavari 1971; Ruebelt 1970). This is believed to be a result of the limited range of hydrocarbons considered in this study. Quantitation was based on gas-chromatographic response for *n*-alkanes up to C_{21}. Many studies consider hydrocarbons up to C_{37} or higher (Ruebelt 1970) or include quite soluble compounds as well as aromatic "envelopes" from oil pollution (Jeltes and den Tonkelaar 1972; Carlberg and Skarstedt 1972; Brown and Huffman 1976; Keizer et al. 1977). Our figures are based only on alkanes and alkanes derived from alkenes. Generally comparable levels of C_{18}–C_{33} *n*-alkanes have been reported for the Clyde estuary and vicinity (Whittle et al. 1974) but similar *n*-alkanes seem to be less at 65–290 ng/ℓ in Bedford Basin, and less still at 78–99 ng/ℓ on the Scotian Shelf (Keizer et al. 1977).

A maximum in the level of lipids may be reached in March and April (Table 1), corresponding to a heavy runoff from the watershed and the related break-up of the ice cover. Traditionally, it is accepted that the latter process leads to the spring algal bloom (cf. chlorophyll decline cited by Schultz and Quinn 1974). An additional high lipid level in August was undoubtedly the result of the passage of hurricane Beth near the coast of Nova Scotia 5 days prior to the sample collection. The surface water of Jeddore Harbour, the basis of all our samples, was heavily laden with sediment for more than 2 wk after this event (August 1971) and lipid extracts were intensely pigmented. The maximum hydrocarbon content

TABLE 1. Recovered lipid and unsaponifiable matter contents (gravimetric determinations) and C_{14}–C_{21} hydrocarbon content (calculated from internal standard) of Jeddore Harbour water. ND = not determined.

Sampling date	Lipid ($\mu g/\ell$)	Unsaponifiable matter ($\mu g/\ell$)	*n*-Alkanes ($\mu g/\ell$) C_{14}–C_{21}
Nov. 10, 1970	ND	10	ND
Dec. 21, 1970	16	9	5
Jan. 27, 1971	30	9	7
Feb. 15, 1971	35	7	1
Mar. 10, 1971	390	23	10
Apr. 19, 1971	160	5	3
May 25, 1971	83	2	2
June 21, 1971	70	6	4
July 15, 1971	65	18	8
Aug. 24, 1971	140	20	10
Sept. 24, 1971	63	2	1
Oct. 20, 1971	93	7	5
Nov. 25, 1971	35	ND	ND
Dec. 23, 1971	88	ND	ND

TABLE 2. Maximum content of C_{14}–C_{22} fatty acids, in hydrogenated form, calculated as recovered from Jeddore Harbour water, in micrograms per litre. I = iso; AI = anteiso; OCFA = odd-chain fatty acids. Note: November was not calculated due to absence of hydrocarbon data.

Fatty acids	Date										
	1970 Dec.	1971 Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.
14:0	0.84	1.1	4.1	39	14	5.9	5.7	6.6	14	5.9	7.3
I15	0.02	—	0.54	2.6	—	0.97	0.71	0.37	1.6	0.60	0.69
AI15	0.02	—	0.17	2.5	—	0.89	0.71	0.33	1.2	0.31	0.34
15:0	0.26	1.1	2.0	14	2.5	0.65	2.3	2.2	6.1	1.5	1.5
I16	—	—	0.17	2.6	0.62	0.24	0.32	—	—	0.06	—
16:0	1.3	1.0	7.8	147	49	13	22	19	57	22	29
I17	0.13	—	—	4.8	—	0.16	—	0.05	0.74	0.06	0.17
AI17	0.02	—	0.11	1.8	31	0.16	1.3	0.19	0.62	0.31	0.17
17:0	0.12	1.8	0.71	6.6	5.3	0.32	1.0	0.89	3.0	0.55	0.86
I18	0.07	—	0.17	—	0.62	—	—	0.09	—	—	0.09
18:0	0.90	2.4	3.7	88	21	20	11	8.5	28	16	30
19:0	0.09	—	0.37	0.73	0.78	—	—	0.05	0.25	—	0.17
20:0	0.48	2.0	1.0	13	6.7	3.9	2.1	1.5	3.2	2.7	5.6
21:0	—	—	—	—	1.1	—	—	0.09	—	0.06	0.09
22:0	—	2.8	—	—	2.5	—	1.0	0.56	3.0	1.4	3.9
OCFA	0.47	2.9	3.1	21	9.6	0.97	3.4	3.0	9.3	2.1	2.7

found in the unnaturally high level in August was of the same order of magnitude as that found in early spring.

The fatty acid composition of water lipids was complicated by the presence of large amounts of unknown components representing from 5 to 40% of total GLC peak areas. A component identified by GLC data only as di-*n*-butyl-phthalate contributed 1–30% of this total. Phthalates have previously been observed in seawater (Pastorelli and Chiavari 1971; Larsson et al. 1974) and presumably are concentrated from the environment into biological samples (Zitko 1973). Analytical blanks and controls conducted during this study confirmed their origin in the seawater, usually with some augmentation in the laboratory except when extraordinary precautions were taken. Fatty acid compositions were thus calculated as normalized percentages based on the recognized odd- and even-chain fully saturated fatty acids, including those increased by hydrogenation, from C_{14} to C_{22} (Table 2). The listing of 20:0, for example, could be due to this acid (Ackman and Hooper 1970), or be partially due to the eicosapentaenoic acid plentiful in unicellular algae (Ackman et al. 1968).

Shorter chain fatty acids C_{14}–C_{18} normally predominate in Jeddore Harbour water and make up 85–95% of the total fatty acids in most samples (Table 2). This may be a reflection of the facile breakdown by autoxidation of long-chain polyunsaturated fatty acids typical of lipids of marine origin (Wagner 1969; Schultz and Quinn 1972). An alternative explanation is that input of lipids is primarily of a plant (Ackman et al. 1968; Hayashi et al. 1974) or plant-derived (Schultz and Quinn 1973) nature, and thus primarily of C_{16} or C_{18} fatty acids. Odd-chain fatty acids appear to occur in water at somewhat higher proportions than those usually encountered in higher living organisms (Ackman and Sipos 1965), possibly because OCFA tend to occur in microbial lipids or be derived from *Zosteria* OCHC by microbial attack. In unhydrogenated esters of fatty acids, 20:0 was occasionally important as reported earlier for oceanic water (Ackman and Hooper 1970; Vityuk 1967). The definite proportions of C_{15} and C_{17} iso and anteiso acids (Table 2) cannot originate from lipids of plants. Although conceivably originating in invertebrate animals (Paradis and Ackman 1976a), there is little doubt that they are also basically of microbial origin, likely from the bacteria flourishing on the freely available detritus (Schultz and Quinn 1973; Gosselink and Kirby 1974).

Variations in relative and absolute levels of OCFA are best examined in Fig. 1. These vary from 2% to 24% of total fatty acids and appear to reach a relative maximum during midwinter and maximum absolute content during early spring. The spring maximum is more a reflection of the very high lipid levels in March and April and may be of limited significance. The winter maximum occurs at a very low quantitative level but may be a reflection of the same factors that produce high OCFA levels in Jeddore Harbour smelt (see below). It should be remembered at the same time that inexplicable variations in the common even-chain fatty acids are great: C_{14}, 10–20%; C_{16}, 30–50%; and C_{18}, 20–40%. In

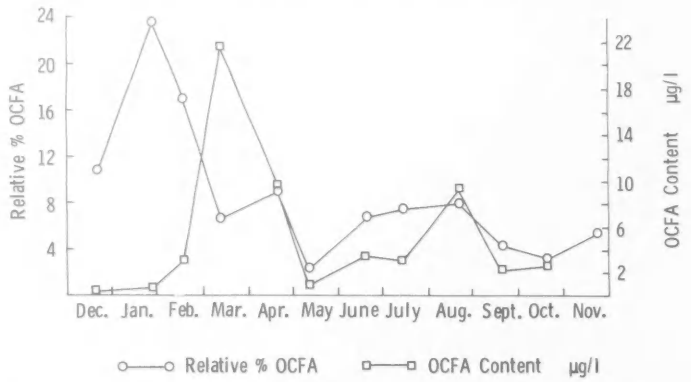

FIG. 1. Changes in monthly levels of total odd-chain fatty acids in Jeddore Harbour water.

TABLE 3. Content of C_{14}–C_{21} straight-chain hydrocarbons, recovered from Jeddore Harbour water, in micrograms per litre. ND = not determined; OCHC = odd-chain hydrocarbons.

Hydro-carbons	Date 1970 Nov.	Dec.	1971 Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct
C_{14}	0.01	0.05	0.15	0.07	0.97	0.22	0.05	0.10	ND	ND	0	0.02
C_{15}	0.33	0.10	0.25	0.12	1.1	0.47	0.13	0.27	0.09	0.40	0.02	0.15
C_{16}	0.12	3.5	0.78	0.23	1.5	0.71	0.26	1.4	0.54	1.4	0.07	0.44
C_{17}	0.26	0.37	1.5	0.27	2.4	0.78	0.35	1.1	1.5	1.8	0.15	1.0
C_{18}	0.15	0.41	3.5	0.29	3.1	0.59	0.68	0.92	3.4	2.0	0.36	1.2
C_{19}	0.24	0.48	0.36	0.10	0.58	0.33	0.14	0.57	1.3	1.8	0.10	0.92
C_{20}	0.13	0.14	0.25	0.05	0.18	0.20	0.13	ND	0.83	1.1	0.08	0.69
C_{21}	ND	0.05	ND	0.06	ND	ND	ND	ND	0.80	1.1	0.08	0.60
ΣOCHC	0.83	1.1	2.1	0.55	4.1	1.6	0.62	2.0	3.7	4.4	0.35	2.7

the confines of a body such as Jeddore Harbour natural factors such as intertidal flushing of harbor waters by varying inputs of freshwater, extent of ice formation during winter and melt-off during the spring, as well as rare phenomena such as hurricane Beth, will cause fluctuations in lipid content or composition more radical than those found in the open sea.

Absolute contents of *n*-alkane (Table 3) also show large variations in levels of all individual components. However odd-chain hydrocarbons (OCHC) occur in amounts roughly equal to even-chain hydrocarbons with the average OCHC level over the 12-mo period being 44% of the total alkanes (cf. Koons et al. 1965).

The distribution of components is reminiscent of that found in diesel oils (Paradis and Ackman 1975) with maximum levels in the intermediate length (C_{17}–C_{19}) components. Table 3, which would be expected to show most clearly any remarkable seasonal variations, shows only mild fluctuations in proportions of particular components. Figure 2 illustrates the characteristic irregular fluctuations in both the relative and absolute levels of OCHC. Pristane is the dominant saturated isoprenoid hydrocarbon in the marine milieu (Ackman 1971), but it is not clearly established that this is derived from zooplankters (Whittle et al. 1974). Other than in waters of the Clyde estuary itself these authors show pristane as about 1/30th of total *n*-alkanes, but in the estuary the proportion was 1/7th. Jeddore Harbour pristane actually averaged 1/30th of the total *n*-alkanes of Table 3. Generally our findings for pristane are qualitatively similar to those reported by Keizer et al. (1977) for geographically adjacent waters.

The virtual disappearance of pristane and phytane from Jeddore Harbour water in the winter (Table 4) was unexpected. The ice cover in this period effectively eliminates operation by pleasure craft and fishing boats, leading to the surmise that these two hydrocarbons could arise from local light oil pollution. Ratios of 0.9–1.5 were found for these two hydrocarbons in diesel oils commonly used in the area (Paradis and Ackman,

TABLE 4. Ratios of two isoprenoid hydrocarbons to each other and to adjacent normal hydrocarbon peaks in gas-chromatographic analysis. NSA = no significant amount.

Proportions of	Date										
	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.
Pristane[a]: phytane[b]	1.15	0.82	NSA	NSA	NSA	NSA	1.37	1.25	1.05	0.67	0.73
n-C_{17}: pristane	1.63	2.93	—	—	—	—	2.57	4.69	4.20	9.86	4.45
n-C_{18}: phytane	1.07	2.65	—	—	—	—	7.15	4.56	9.90	2.93	8.07

[a]2, 6, 10, 14-tetramethylpentadecane.
[b]3, 7, 11, 15-tetramethylhexadecane.

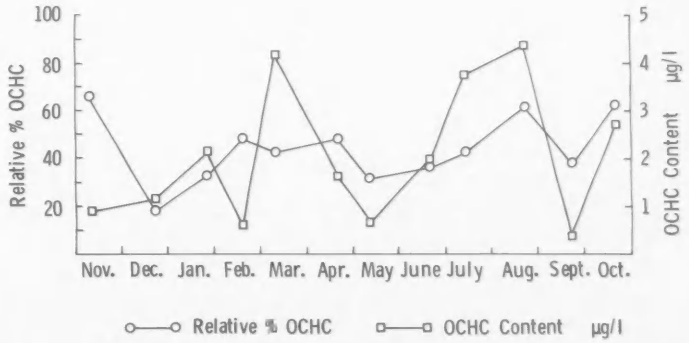

FIG. 2. Changes in monthly levels of total odd-chain hydrocarbons in Jeddore Harbour water.

1975), and our ratios fit an origin in petroleum (Brooks et al. 1969). Unfortunately the meaningfulness of this ratio has been challenged (Hellman and Holeczek 1974). The higher chain lengths ($>C_{21}$) found by Barbier et al. (1973) to be more important in offshore waters than in coastal waters were absent. Iliffe and Calder (1974) indicate n-alkanes $<C_{21}$ to be generally important in the heavily traveled and industrialized Gulf of Mexico. The ratios of n-alkanes to the isoprenoids increase slowly from May through September, suggesting that n-alkanes of biotic origin may be added to a base hydrocarbon composition, probably orginating in local pollution. Moreover, there is no marked drop in total unsaponifiable material in the winter samples (Table 1) so the pollution-based hydrocarbon must be almost totally replaced, probably by biotic hydrocarbons from decaying vegetation.

The seasonal survey (Fig. 1, 2) indicates that both fatty acids and hydrocarbons are present in Jeddore Harbour water in amounts comparable to those found in other studies on marine estuarine samples. Neither hydrocarbons, total lipids, or fatty acids show unexpected characteristics. There is no evident correlation between OCHC content and composition and unusually high levels of OCFA. A correlation may, however, possibly exist between OCFA levels in water (Fig. 1) and a biological source. The relative amount of OCFA's appear to reach a maximum during the winter and may reflect the similar changes that occur during the winter in the lipids of smelt. These have now been localized to an origin in the amphipod *Pontoporeia femorata* (Paradis and Ackman 1976a, b). There are unusually high concentrations of *P. femorata* in Jeddore Harbour and a proportion, mostly males, enter the water column in December–January. Since their lipid fatty acids include about 50% OCFA, natural mortality will lead to a substantial effect on water fatty acid composition at this time when input of fatty acids from other sources is minimal.

The increased runoff into Jeddore Harbour from hurricane Beth cannot be more than estimated, but in 24 h approximately 28 cm of precipitation fell in the general area, compared to the 7–11 cm normal for individual months in the July–September period for the area. In small streams heavy runoffs from events such as hurricanes disturbs deposited material and the macrobenthic community, although the status quo is usually quickly restored (Hoopes 1974). Unexpectedly we found that the impact of hurricane Beth on lipid compositional details was surprisingly small. Possibly this was because the warm

fresh runoff water would overlay the colder salt water and would be rapidly discharged without altering nutrients or other factors in the salt water originating primarily from the open ocean and replenished at each tide. Alternatively, the runoff water, not coming from an industrialized or urban area, would have natural alkanes, fatty acids, and other lipids not differing much in detail from those of natural marine origin normally found in Jeddore Harbour.

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Leucocrit: A Simple Hematological Technique for Measuring Acute Stress in Salmonid Fish, Including Stressful Concentrations of Pulpmill Effluent

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MCLEAY, D. J., AND M. R. GORDON. 1977. Leucocrit: a simple hematological technique for measuring acute stress in salmonid fish, including stressful concentrations of pulpmill effluent. J. Fish. Res. Board Can. 34: 2164–2175.

The number of leucocytes and thrombocytes in the circulating blood of fishes seems to reflect more accurately than the number of erythrocytes the fish's reaction to acute stress, including that caused by pollution. To facilitate the estimation of leucocytes in fish blood, a new test is being introduced called leucocrit, which is based on the determination of the volume of packed leucocytes–thrombocytes expressed as a percentage of the whole blood. For stocks of juvenile coho salmon (*Oncorhynchus kisutch*) and rainbow trout (*Salmo gairdneri*) held in freshwater under a variety of conditions, mean leucocrit values varied from 0.55 to 1.91%. Variations in age, species, food ration, degree of crowding, and prior disturbances of fish stocks were associated with these differences. Leucocrit and leucocyte–thrombocyte counts for both species were depressed from stock values after 96-h or shorter (rainbow) exposure to stressful (high-temperature crowded) conditions; values for these measures were positively correlated but uncorrelated with hematocrit values. Hematological values for subpopulations of underyearling rainbow trout reared under summer or winter photoperiod conditions were unaffected by photoperiod and responded similarly to acute stress. Leucocrit values for stocks of coho ($1.30 \pm 0.27\%$) and rainbow ($0.92 \pm 0.12\%$) were depressed due to transfer or acute exposure to bleached kraft pulpmill effluent (BKME). A 24-h exposure to BKME after 2 days' acclimation caused consistent leucocrit depressions, with median effective concentrations of 0.28 of the 96-h LC50 value for coho and 0.16 LC50 for rainbow. Hematocrit showed less sensitive or consistent changes due to stress or effluent exposure than leucocrit.

Key words: leucocrit, leucocyte–thrombocyte count, hematocrit, blood, acute stress, BKME, pulpmill effluent, sublethal, salmonid fish, applications

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Le nombre de leucocytes et de thrombocytes dans le sang en circulation des poissons semble refléter avec plus de précision que le nombre d'érythrocytes la réaction des poissons à un stress aigu, y compris celui causé par la pollution. Afin de faciliter l'estimation des leucocytes dans le sang des poissons, nous introduisons un nouveau test appelé *leucocrite*, fondé sur la mesure du volume de leucocytes–thrombocytes concentrés, exprimé en pourcentage du sang entier. Pour des stocks de saumons coho (*Oncorhynchus kisutch*) et de truites arc-en-ciel (*Salmo gairdneri*) maintenus en eau douce dans une variété de conditions, les valeurs moyennes du leucocrite varient de 0.55 à 1.91%. Associées à ces différences sont les vraiations dans l'âge, l'espèce, la ration alimentaire, le degré de surpeuplement et les dérangements antérieurs des stocks de poissons. Le leucocrite et le nombre de leucocytes–thrombocytes chez les deux espèces diminuent par rapport aux valeurs des stocks après des expositions de 96 h ou moins (truite arc-en-ciel) à des conditions de stress (surpeuplement à des températures élevées); ces mesures montrent une corrélation positive entre elles, mais elles n'ont pas de corrélation avec les valeurs de l'hématocrite. Les valeurs hématologiques observées dans les sous-populations de truites arc-en-ciel de moins d'un an élevées dans des conditions de photopériode estivale ou hivernale ne sont pas affectées par la photopériode et réagissent de la même façon à un stress aigu. Les valeurs du leucocrite pour des stocks de coho ($1.30 \pm 0.27\%$) et de truites arc-en-ciel ($0.92 \pm 0.12\%$) diminuent à la suite d'un transfert ou d'une exposition aiguë aux eaux usées de blanchiment de papeterie kraft (BKME). Une exposition de 24 h au BKME après 2 jours d'acclimatation produit des diminutions uniformes du leucocrite, avec concentrations effectives médianes de 0.28 de la

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valeur de la CL50 après 96 h pour le saumon coho et de 0.16 CL50 pour la truite arc-en-ciel. L'hématocrite subit des changements moins sensibles ou uniformes que le leucocrite sous l'influence d'un stress ou d'une exposition aux eaux usées.

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HEMATOLOGICAL techniques, including measurements of hematocrit, erythrocyte and leucocyte counts, have proven valuable for fisheries biologists in assessing the health of fish (Blaxhall 1972; Bell and Margolis 1976; Hickey 1976) and in monitoring stress responses (Swift and Lloyd 1974; Soivio and Oikari 1976), including those due to exposure to sublethal strengths of toxicants (McKim et al. 1970; Bills and Hunn 1976; Iwama et al. 1976). In studies designed to determine stressful concentrations in kraft pulpmill wastes to salmon, we determined that acute exposure to dilute sublethal concentrations causes marked decreases in the numbers of leucocytes (white blood cells) and/or thrombocytes (WBC-T counts) within the peripheral blood of juvenile coho salmon (*Oncorhynchus kisutch*) (McLeay 1973a, 1975a; McLeay and Howard 1977). Because of the sensitivity, reliability, and rapidity of this response and its apparent involvement in disease resistance of fishes (Watson et al. 1956; Corbel 1975), we developed and evaluated a simple, rapid alternative procedure for determining the numbers of circulating leucocytes–thrombocytes. This procedure, termed here as "leucocrit," is similar to the microhematocrit procedure for rapid assessments of the numbers of circulating erythrocytes (Hesser 1960; Snieszko 1960).

This paper describes experiments conducted to standardize this technique and to assess its usefulness in estimating the WBC-T counts, in determining the condition of fish stocks, and in detecting early stress, including that caused by exposure to sublethal concentrations of kraft pulpmill effluents. Leucocrit and hematocrit values are presented and compared for both coho salmon and rainbow trout (*Salmo gairdneri*).

Materials and Methods

FISH AND WATER SUPPLY

The coho salmon tested as yearlings or underyearlings were obtained as swim-up fry (Capilano Hatchery, British Columbia) or eyed eggs (Qualicum Hatchery, British Columbia), respectively, from the Department of Fisheries and the Environment. An additional population of coho sampled as 2-yr fish in freshwater was received as swim-up fry from the Washington State Department of Fisheries (Skagit Hatchery). Rainbow trout were received as fry from Troutlodge Inc. (Washington State) on three occasions. All fish groups were held at B.C. Research in dechlorinated Vancouver city tap water (temperature 11.0 ± 1.5°C, pH 6.5 ± 0.2, dissolved oxygen 10.9 ± 0.4 mg/ℓ, alkalinity 5.0 ± 0.9 mg $CaCO_3$/ℓ, EDTA hardness 5.6 ± 0.9 $CaCO_3$/ℓ, conductivity 15.8 ± 1.6 μmhos/cm). Fish were fed an Oregon Moist Pellet ration (Moore-Clark Ltd.; La Conner, Washington) and maintained according to standard hatchery practice. Overhead incandescent illumination for fish groups held indoors was regulated by photocell or timer, as indicated, and was relayed through 30-min dimmer–brightener rheostats to provide a dawn–dusk effect.

TECHNIQUES AND EFFECTS OF PROCEDURAL VARIATIONS

Blood collection and analytical techniques — Fish were sampled by rapidly netting individuals, severing their caudal peduncle, and drawing the blood into a narrow-bore heparinized microhematocrit tube (0.55 ± 0.05 mm ID; Sherwood Industries Ltd., St. Louis). Tubes were sealed and centrifuged at 11500 rpm (13960 *g* force) for 3 or 5 min. Blood samples were measured for leucocrit and hematocrit values within 5 min of centrifugation. Leucocrit was determined by measuring the height of the buffy layer (grayish-white layer of cells separating the erythrocytes from the plasma) to the nearest 0.02 mm using a dissecting microscope (50× magnification) and ocular micrometer. Each tube was rotated during measurement, and the average height of the buffy layer was approximated. The leucocrit value, defined as the volume of packed leucocytes and thrombocytes as a percentage of the whole blood volume, was calculated as follows: height of buffy layer ÷ height of total blood volume × 100 = % leucocrit. Hematocrit (volume of packed erythrocytes as percentage of total blood) was determined according to Snieszko (1960).

Blood portions analyzed for WBC-T counts were drawn directly from the severed peduncle into a blood dilution pipette immediately after sampling the same fish for leucocrit–hematocrit determinations. These samples were mixed with Rees-Ecker diluting fluid (Klontz and Smith 1968). Using a hemacytometer and a compound microscope with oil-immersion lens, a pooled count of leucocytes and thrombocytes (McLeay 1975a) was determined for each stained solution.

Contents of buffy layer — Fresh blood from 10 underyearling coho salmon was centrifuged in heparinized microhematocrit tubes for 5 min. Smears of

the cellular constituents of the buffy layer were prepared and stained by the Leishman-Giemsa technique (Klontz and Smith 1968). Differential cell counts were carried out for each at 1000× magnification; 100 cell types were classified as to WBC-T, erythrocyte or other (unidentified).

Effect of interval between blood collection and centrifugation — Twelve yearling coho salmon (wt 9.16 ± 5.38 g, length 8.8 ± 1.7 cm) were netted individually from an indoor 1000-ℓ tank, and blood collected within 45 s. For the initial six fish sampled, the first blood tube filled was centrifuged immediately, while the second, third, fourth, and fifth tubes were centrifuged at 3, 6, 9, and 12 min after sampling, respectively. For the remaining six fish sampled, the first tube was again centrifuged immediately but the fifth tube filled was spun at 3 min, the fourth tube at 6 min, the third at 9 min, and the second at 12 min. The tubes were held vertically in a sealing putty tray at 11°C until centrifuged (3 min). Leucocrit and hematocrit values were determined immediately thereafter. A two-way analysis of variance was used to assess the effects of elapsed time and successive blood samplings on the leucocrit and hematocrit values measured from the same fish.

Effect of interval between centrifugation and measurement — Twelve underyearling coho salmon (5.35 ± 1.69 g, 7.6 ± 0.7 cm) and 12 rainbow trout (2.90 ± 0.88 g, 6.5 ± 0.5 cm) were sampled individually from fish stocks held outdoors in separate 6500-ℓ fiberglass tanks. One tube of blood was drawn from each fish and centrifuged (5 min) immediately. Leucocrit and hematocrit measures were then recorded immediately following centrifugation and at 0.25, 0.5, 1.0, 3.0, and 24 h thereafter. Between measurements the centrifuged blood samples were held vertically at 11°C.

Variations in Stock Values

A cursory examination was conducted to gain some insight into the extent to which fish species (coho or rainbow only), developmental stage, and rearing conditions affected leucocrit and hematocrit values. During a 5-mo period, eight groups of coho salmon and nine of rainbow trout (n = 12–15) varying in age from 3 to 31 mo and held in stock tanks under diverse conditions were sampled directly from these tanks for leucocrit and hematocrit determinations. The varied rearing conditions included outdoor vs. indoor tanks; excess (5% of wet wt, 3 times daily) vs. limiting (5% of wet wt, once daily) rations; crowded vs. uncrowded; and frequently disturbed vs. undisturbed states. For each group, blood collection, centrifugation, and measurements of hematocrit and leucocrit were completed within 15 min. Lengths and weights for each fish were measured subsequently.

Acute Stress and Photoperiod Effects

Underyearling coho salmon were held indoors in a 1000-ℓ fiberglass tank under simulated natural photoperiod conditions for 10 mo. At the time of this study the fish weighed 8.19 ± 1.51 g and measured 9.0 ± 0.8 cm fork length. A group of 15 fish was placed in a 50-ℓ capacity rectangular polyethylene tank (45 × 35 × 40 cm) containing the minimum amount of water sufficient to permit respiration but not enough to cover dorsal fins or permit free swimming (McLeay 1975a). The water was kept oxygen saturated by vigorous aeration. Temperature was maintained at ambient laboratory temperature (20 ± 1.3°C) and illumination was constant. All fish were transferred to a fresh volume of water every 24 h to minimize contamination from metabolic wastes. This test group was held unfed in this condition for 4 days, followed by sampling of all fish for leucocrit, hematocrit, and WBC-T determinations. At this time, a group of 15 fish (unfed for 24 h) was also sampled directly from the stock tank for comparison of values with the stressed group.

In a second study, two subpopulations of 1000 underyearling rainbow trout were held indoors in separate 1000-ℓ fiberglass tanks under photoperiod regimes of 16 h light:8 h dark (16L:8D) or 8 h light:16 h dark (8L:16D). Feeding rations for each fish stock were identical. After 13 wk of acclimation to summer or winter photoperiods, five groups of 15 fish each were selected randomly from each stock tank and transferred to separate polyethylene tanks (15 fish/tank) where they were exposed to high temperature and minimal water conditions identical to those described for coho salmon. All fish within each group were killed for leucocrit and hematocrit determinations at intervals of 6, 24, 48, 72, or 96 h following transfer to these stressful conditions. A group of 15 fish was also sampled directly from each stock tank. Fish acclimated to the summer photoperiod weighed 3.60 ± 0.84 g and measured 6.7 ± 0.5 cm at the time of this study, while those held under a winter photoperiod were 3.53 ± 1.05 g and 6.6 ± 0.6 cm. Analytical procedures were those described for coho salmon except that the WBC-T counts were determined only for the stock samples and for fish stressed for 96 h.

The correlations of WBC-T, leucocrit, and hematocrit values measured from the same fish were determined for the stock and 96-h stressed samples of each species. Correlation coefficients (r values) for WBC-T: leucocrit, WBC-T: hematocrit, and leucocrit: hematocrit comparisons were examined for significance according to Student's t-statistic.

Response to Sublethal Concentrations of Pulp-mill Effluent

Acclimation and BKME effects — Ten groups of 12 underyearling coho salmon (wt 2.10 ± 0.68 g, length 6.1 ± 0.6 cm) and 10 groups of 12 rainbow trout fingerlings (wt 1.19 ± 0.36 g, length 5.2 ± 0.5 cm), held in outdoor 6500-ℓ tanks for 7 wk, were transferred to separate rectangular polyethylene tanks (12 fish/tank) containing 44 ℓ of freshwater. Each tank received a continuous supply of 11 ± 0.5°C water at a rate that caused 95% turnover within 3 h. Aeration to each tank was minimal through glass Pasteur pipettes. Each tank was fitted with a translucent polyethylene lid to minimize disturbance to

the fish. Overhead incandescent lighting was regulated by a photocell, with a 30-min dawn–dusk period of varying intensity. Test fish were not fed 16 h prior to transfer nor during the acclimation or exposure periods.

After acclimation periods of 1, 2, 3, 5, or 7 days, the water supply to two tanks was shut off. A measured volume (9.2 ℓ) of water was siphoned from each and was replaced with the same volume of freshwater (control tank) or bleached kraft mill effluent (BKME) providing an effluent concentration equivalent to 0.8 of the 96-h median tolerance limit (LC50) for each species. Siphoning and replacement of water or BKME solutions using Tygon tubing inserted through a hole in the lid caused a minimum of disturbance to the fish. After a 24-h exposure period, all fish within the control and effluent-exposed tanks were netted individually, their blood collected and centrifuged immediately, and leucocrit and hematocrit measured directly thereafter. Twelve fish of each species were similarly sampled directly from the stock tanks just prior to transfer of groups to the test aquaria and at the termination of the study.

The effluent used here and for the LC50 bioassays was a single grab sample of whole mill effluent obtained during normal operations from a British Columbia coastal mill employing primary clarification treatment only. The wood furnish was 40% hemlock, 10% fir, and 50% cedar, and the bleaching sequence employed was CEHDED (chlorination, caustic extraction, hypochlorite, chlorine dioxide, caustic extraction, and chlorine dioxide). Characteristics included pH 3.0, conductance 1250 μmhos/cm, sodium 196 mg/ℓ, color 1776 APHA units, total organic carbon 230 mg/ℓ, BOD_5 199 mg/ℓ, and chemical oxygen demand 724 mg/ℓ. The sample was transported in full, sealed 200-ℓ polyethylene containers; mixed and filtered to remove fiber; and stored at 7°C in full, sealed containers. As required, portions for bioassays were heated to 11°C, adjusted to pH 6.5 (that of the dilution water) using 1 N NaOH and H_2SO_4, and aerated to saturation level. The toxicity of this effluent according to conventional 96-h LC50 bioassays (APHA et al. 1975) was determined for the stock supply of each species at the time of the study, using 10 fish per concentration. After a 48-h acclimation to the polyethylene tanks, fish were exposed to 2 ℓ effluent·g $fish^{-1}$·day^{-1}, with 95% replacement of test solutions every 24 h. Test conditions for the LC50 bioassays were identical to those described for the blood tests.

The differences in the leucocrit and hematocrit values between each effluent-exposed and corresponding control group were compared for significance using Student's *t*-test (variances not assumed to be homogeneous). These tests were one-tailed for the leucocrit data and two-tailed for the hematocrit data.

Sensitivity to BKME — In an initial study nine groups of 15 coho salmon (1.79 ± 0.60 g, 5.6 ± 0.4 cm) and the same for rainbow trout (1.24 ± 0.28 g, 5.0 ± 0.3 cm) were transferred from outdoor stock tanks to the test tanks (15 fish/tank). After a 48-h acclimation period, the water flow to each tank was discontinued and fish were exposed to BKME (sample used in previous study) concentrations equivalent to 0.025, 0.05, 0.1, 0.2, 0.4, 0.6, or 1.0 of the 96-h LC50 value for each species (based on bioassays conducted concurrently). Exposures were staggered at 20-min intervals to provide sufficient time for sampling. Test conditions for the blood and LC50 bioassays were identical to those described in the previous study. Two control tanks had volumes of water replaced equivalent to that for the lowest and highest effluent concentrations. After a 4-h exposure to effluent, all fish were killed for leucocrit and hematocrit determinations according to the procedure used formerly. Fifteen fish of each species were sampled directly from the stock tanks.

A similar study using the same fish stocks was conducted 4 wk subsequently; the major procedural variation being duration of exposure to effluent. Groups of 12 fish of each species (2.28 ± 0.58 g and 6.1 ± 0.5 cm coho; 1.45 ± 0.39 g and 5.4 ± 0.5 cm rainbow) were transferred to separate tanks. After 48 h, the fish groups were exposed to BKME concentrations equivalent to 0.0 (two control tanks), 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 of the 96-h LC50 (determined concurrently). Additionally, two groups of each species were exposed to a portion of this effluent sample that had been previously detoxified (full-strength effluent not lethal within 96-h) by 5-day aerated lagoon treatment under controlled laboratory conditions (Mueller and Walden 1974). The two concentrations of treated effluent to which fish were exposed were 90% by volume and that concentration equivalent to 1.0 LC50 for untreated effluent. Effluent additions were staggered at 20-min intervals and all fish were killed for leucocrit and hematocrit analyses after a 24-h exposure period. At the initiation of this study, a group of 12 fish was also sampled directly from each stock tank.

The effluent used for the LC50 and blood bioassays in the second (24-h exposure) study was a grab sample of whole mill effluent collected during normal operations from the same mill as the previous sample tested. Wood furnish was 58% hemlock, 27% fir, and 15% cedar, and the bleaching sequence was CEHDED. Effluent characteristics were pH 3.2, sodium 245 mg/ℓ, conductance 1500 μmhos/cm, color 1330 APHA units, total organic carbon 306 mg/ℓ, BOD_5 237 mg/ℓ, and chemical oxygen demand 768 mg/ℓ. This BKME sample was handled as per the initial sample.

In each study the leucocrit and hematocrit values for the two control groups were pooled, since data did not differ significantly according to Student's *t*-test. Values for each effluent-exposed group were tested for significance against the pooled control data using one- (leucocrit) or two-tailed (hematocrit) Student's *t*-tests.

In the 24-h exposure study, the median effective concentration (EC50) of BKME causing a decrease in leucocrit was estimated for each species. The proportion of fish at each concentration showing a net significant decrease in leucocrit (values less than the lower 95% confidence limit for controls) was determined; EC50 values and their confidence limits were

then calculated by log-probit analysis (Finney 1971; APHA et al. 1975).

Results

Contents of Buffy Layer

Differential cell counts of buffy layer smears from centrifuged blood of coho salmon revealed the following: combined counts of leucocytes and thrombocytes, 95.3 ± 1.9 %; erythrocytes, 3.2 ± 1.2 %; and unidentified cells, 1.5 ± 1.0 % (mean ± SD values for 10 fish). Thus, the buffy layer comprises mainly leucocytes and thrombocytes, with a minor contamination by erythrocytes.

Effects of Procedural Variations

According to a two-way analysis of variance, leucocrit values were not affected significantly by elapsed time prior to centrifugation nor by the order of sample withdrawal from the same fish (Table 1). Variation of hematocrit values was also unaffected by the successive order of blood collection; however, the increase with time of mean values for both fish groups (Table 1) caused significant variation ($P < 0.05$).

Leucocrit values for coho salmon and rainbow trout were stable during the 1st h after centrifugation (Table 2); however, mean values for both species were increased somewhat at 3 h and increases were significant at 6 and 24 h. Although the hematocrit values were not altered significantly for either species within 24 h of standing, mean values showed a trend towards increasing at 6 and 24 h (Table 2).

Variations in Fish Stocks

Variations in mean leucocrit values for the groups of coho salmon or rainbow trout sampled directly from stock tanks were threefold, whereas the extent of changes in hematocrit values was much less (Table 3). For both species leucocrit values appeared to be affected by the stage of development. The youngest (<1g) fingerlings had values considerably below those of the older groups sampled. Mean leucocrit values increased for both species within 1 wk of increasing their food ration; however, thinning of the rainbow trout population during this interval might have contributed to this response. Disturbances to fish stocks prior to sampling by frequent nettings of other fish within the same tank were associated with decreases in leucocrit values for both species; hematocrit values for the same fish were not altered appreciably. Rearing either species indoors or outdoors under otherwise similar conditions did not change leucocrit or hematocrit values to any measurable extent. Leucocrit values for coho salmon were consistently higher than those for rainbow trout similar in size, stage of development, and rearing conditions (Table 3). Other possible influences, such as fish size or condition factor, population density or photoperiod, were not clearly evident.

Acute Stress and Photoperiod Effects

The WBC-T counts and leucocrit values for coho salmon and both groups of rainbow trout were depressed significantly from stock values after 96 h of stress (Table 4). Leucocrit values for rainbow trout reared under summer or winter photoperiod regimes were also significantly depressed with shorter (6, 24, 48, and 72 h) durations of stress; the response being greatest at 6 h. The WBC-T and leucocrit values for both stock and 96-h stressed groups of rainbow trout were less than corresponding values for coho salmon. Hematocrit values for both subpopulations of rainbow trout were elevated after exposure to stressful conditions for 6, 24, 48, 72, or 96 h, while values for coho salmon were unchanged by 96 h of stress. The different photoperiod regimes under which the rainbow trout were reared did not alter the blood values measured nor the manner in which they responded to stress.

The WBC-T and leucocrit values determined for the same fish were significantly ($P < 0.01$) and positively correlated for all stock and 96-h stressed samples of each species. Correlation coefficients for all WBC-T:hematocrit and leucocrit:hematocrit comparisons of these same groups were not significant.

Acclimation and BKME Effects

With both species, leucocrit and hematocrit values for the initial and final stock samples did not differ significantly according to Student's *t*-test. The acute lethal tolerance limit of these fish stocks to the effluent sample used in the blood tests was the same (96-h LC50, 25.8% [95% confidence limits 24.0 and 27.8%] for coho and 25.3 % [23.5 and 27.2] for rainbow).

Mean leucocrit values for all groups of coho salmon fingerlings transferred to aquaria and sampled after 2–8 days in freshwater were depressed from stock values, irrespective of the acclimation period (Table 5). Corresponding values for rainbow trout were consistently lower; leucocrit measurements were slightly decreased or unaffected by transfer. Leucocrit values for all groups of coho exposed to pulpmill effluent for

TABLE 1. Effects of time prior to centrifugation and of successive blood samplings on leucocrit and hematocrit values of yearling coho salmon. Values are expressed as means ± SD ($n = 6$).

Interval between blood collection and centrifugation (min)	Leucocrit (%)		Hematocrit (%)	
	Serial sampling order[a]	Reversed sampling order[b]	Serial sampling order[a]	Reversed sampling order[b]
0	1.96±0.62	1.86±0.48	27.0±2.5	27.2±1.5
3	1.89±0.60	1.63±0.47	27.8±1.2	25.5±1.8
6	2.00±0.69	1.86±0.61	27.8±1.3	27.3±1.5
9	1.96±0.73	1.84±0.61	28.2±1.2	29.0±1.1
12	1.72±0.80	1.82±0.51	28.0±1.5	29.8±1.0

[a]First of five tubes of blood drawn from the same fish centrifuged immediately; second, third, fourth, and fifth centrifuged at 3, 6, 9, and 12 min, respectively

[b]First tube centrifuged immediately; fifth, fourth, third, and second centrifuged at 3, 6, 9, and 12 min, respectively.

TABLE 2. Effects of time after centrifugation on leucocrit and hematocrit values of underyearling coho salmon and rainbow trout. Values are expressed as means ± SD value ($n = 12$). P values are based on Student's t-test (variances not assumed to be homogeneous) and represent significance of difference between the initial and subsequent measurements. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Interval between centrifugation and measurement (h)	Leucocrit (%)		Hematocrit (%)	
	Coho	Rainbow	Coho	Rainbow
0	1.73±0.49	1.04±0.25	22.1±2.3	24.4±3.6
0.25	1.76±0.50	1.05±0.24	22.2±2.3	24.5±3.7
0.5	1.79±0.49	1.07±0.23	22.2±2.3	24.5±3.7
1.0	1.83±0.49	1.09±0.24	22.4±2.2	24.6±3.4
3.0	2.06±0.48	1.23±0.24	22.6±2.5	24.7±3.5
6.0	2.22±0.53*	1.33±0.15**	22.9±2.3	25.2±3.5
24.0	2.49±0.53**	1.62±0.32***	23.3±2.2	25.7±3.5

24 h were decreased from corresponding control values ($P < 0.001$) with no apparent influence due to acclimation period. The leucocrit response of rainbow trout to effluent was less pronounced and was only significant for fish groups acclimated to aquaria for 1, 2, or 5 days prior to exposure. Hematocrit values for either species were not altered appreciably by effluent exposure (Table 5).

The median lethal concentrations of BKME for the two salmonid species exposed to a range of sublethal strengths of effluent for 4 or 24 h were as follows (95% CL in parentheses):

	96-h LC50 (%)	
	coho	rainbow
BKME sample no. 1 (4-h exposure test)	22.4 (20.8, 24.3)	18.2 (17.1, 19.2)
BKME sample no. 2 (24-h exposure test)	15.8 (14.6, 17.0)	18.1 (17.1, 19.2)

Since the sublethal concentrations were expressed as portions of the 96-h LC50 value for each fish group, the sensitivity of leucocrit or hematocrit responses was not affected by these slight differences in acute lethal tolerance.

In the initial study to determine the sensitivities of leucocrit or hematocrit responses to pulpmill effluent, leucocrit values for both species were unaffected by a 4-h exposure to BKME concentrations up to and including 1.0 LC50. Hematocrit measurements were unaffected by effluent concentrations, except 0.6 or 1.0 LC50, where numbers were elevated slightly but significantly above corresponding control values (Table 6). In a study conducted 4 wk later under identical conditions other than exposure period, leucocrit values for coho salmon and rainbow trout were significantly depressed by a 24-h exposure to effluent strengths of 0.4–1.0 LC50 (Table 7). The median effective concentrations causing leucocrit depressions were 0.28 LC50 (95% CL, 0.21 and 0.35) for coho and 0.16 LC50 (0.004, 0.30) for rainbow. However, when these fish stocks were exposed for 24 h to biotreated (detoxified) portions of this effluent sample at strengths equivalent to 1.0 LC50 or 90% by volume (5–6 times the LC50 concentration),

TABLE 3. Effects of fish age and rearing conditions on leucocrit and hematocrit values for coho salmon and rainbow trout.

Species	No. of fish	Age (mo)	Month sampled	Length (cm)	Wt (g)	Holding conditions	Leucocrit (%)	Hematocrit (%)
Coho[a]	15	4	June	4.81 (0.55)	0.92 (0.39)	Outdoor 6500-ℓ tank; limiting ration	1.07 (0.24)	28.64 (4.41)
Coho[a]	15	4.25	June	5.57 (0.41)	1.79 (0.60)	Same as above but given excess ration for 1 wk prior to sampling	1.37 (0.37)	27.06 (2.52)
Coho[a]	12	5	July	6.04 (0.56)	2.36 (0.66)	Same as above but given excess ration for 4 wk prior to sampling	1.43 (0.23)	28.83 (1.69)
Coho[a]	12	5.25	July	6.05 (0.47)	2.20 (0.50)	Same as above but disturbed several times during week prior to sampling	1.15 (0.23)	26.92 (1.93)
Coho[a]	15	5.25	July	6.01 (0.61)	2.50 (0.94)	Held under artificial illumination (simulated natural photoperiod)	1.39 (0.20)	26.80 (2.01)
Coho[a]	12	8.25	Oct.	7.63 (0.73)	5.35 (1.69)	Held in outdoor 6500-ℓ tank for 5 mo prior to sampling; fed excess ration	1.73 (0.49)	24.08 (2.27)
Coho[b]	12	19	Oct.	17.10 (1.77)	46.37 (12.10)	Yearling coho held indoors (simulated natural photoperiod) in freshwater in a 1000-ℓ tank for 4 mo prior to sampling	1.91 (0.53)	27.09 (1.97)
Coho[c]	12	31	Oct.	20.80 (1.77)	106.99 (26.27)	2-yr coho held outdoors in freshwater in a 6500-ℓ tank for 4 mo prior to sampling	1.55 (0.23)	24.82 (4.07)
Rainbow[d]	12	3	July	4.32 (0.38)	0.69 (0.21)	Held indoors in hatchery trough for 2 mo prior to sampling	0.55 (0.21)	25.60 (3.09)
Rainbow[e]	15	3.5	June	4.75 (0.25)	0.61 (0.18)	Outdoor 6500-ℓ tank;limiting ration	0.55 (0.15)	28.50 (3.56)
Rainbow[e]	15	3.75	June	4.97 (0.29)	1.24 (0.28)	Same as above but given excess ration and less crowded (population thinned) for 1 wk prior to sampling	0.93 (0.22)	26.46 (2.87)
Rainbow[e]	12	4.5	July	5.34 (0.12)	1.43 (0.20)	As above but given excess ration for 4 wk prior to sampling	0.97 (0.10)	26.66 (2.30)
Rainbow[e]	12	4.75	July	5.43 (0.49)	1.45 (0.39)	As above but disturbed several times during week prior to sampling	0.85 (0.10)	26.92 (2.15)
Rainbow[e]	12	4.75	July	5.21 (0.36)	1.35 (0.32)	Transferred from outdoor 6500-ℓ tank to indoor 1000-ℓ tank 1 mo prior to sampling	0.94 (0.17)	25.13 (2.44)
Rainbow[e]	12	7.75	Oct.	6.48 (0.51)	2.90 (0.88)	Held in outdoor 6500-ℓ tank for 5 mo prior to sampling; crowded conditions	1.04 (0.25)	24.42 (3.62)
Rainbow[e]	12	7.75	Oct.	8.83 (1.68)	9.16 (5.38)	Held indoors under uncrowded conditions in 1000-ℓ tank for 4 mo prior to sampling	1.22 (0.20)	24.33 (2.74)
Rainbow[f]	12	7	July	7.08 (0.72)	3.80 (1.10)	Held indoors in 1000-ℓ tank for 4 mo prior to sampling	1.11 (0.29)	25.50 (3.03)

[a]Received as eyed eggs from Department of Fisheries and the Environment (Qualicum Hatchery) in February 1976; transferred to outside tank in May.
[b]Received as fry from Department of Fisheries and the Environment (Capilano Hatchery) in April 1975.
[c]Received as fry from Washington State Department of Fisheries (Skagit Hatchery) in April 1974.
[d]Received as fry from Troutlodge Hatchery (Washington State) in May 1976.
[e]Received as fingerlings from Troutlodge Hatchery in May 1976.
[f]Received as fry from Troutlodge Hatchery in January 1976.

TABLE 4. Effects of acute stress (crowding and elevated temperature) and photoperiod on WBC-T counts, leucocrit, and hematocrit of underyearling rainbow trout and coho salmon. Values are expressed as means with SD in parentheses ($n = 15$). All P values are based on Student's t-test (variances not assumed to be equal) and represent significance of difference between each stressed group and the corresponding stock sample. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Duration of stress (h)	WBC-T count (×10 = no./mm³)			Leucocrit (%)			Hematocrit (%)		
	16L:8D rainbow[a]	8L:16D rainbow[b]	Coho[c]	16L:8D rainbow	8L:16D rainbow	Coho	16L:8D rainbow	8L:16D rainbow	Coho
0 (stock)	47.1 (11.7)	47.7 (8.7)	55.6 (13.0)	0.78 (0.15)	0.82 (0.16)	1.11 (0.28)	26.5 (2.1)	26.3 (1.5)	25.9 (3.0)
6	—[d]	—	—	0.28*** (0.27)	0.33*** (0.22)	—	37.3*** (4.8)	36.4*** (2.4)	—
24	—	—	—	0.45*** (0.18)	0.48*** (0.23)	—	36.8*** (3.8)	35.8*** (3.5)	—
48	—	—	—	0.50*** (0.27)	0.65*** (0.30)	—	35.3*** (3.4)	35.3*** (2.6)	—
72	—	—	—	0.60*** (0.16)	0.65*** (0.10)	—	33.8*** (4.1)	32.4*** (2.9)	—
96	36.3*** (6.6)	38.9*** (7.1)	44.0*** (8.1)	0.63* (0.18)	0.63** (0.25)	0.85* (0.38)	28.7* (3.3)	32.9*** (2.7)	26.8 (3.2)

[a]Fish acclimated to 16 h light:8 h dark for 13 wk.
[b]Fish acclimated to 8 h light:16 h dark for 13 wk.
[c]Fish acclimated to natural photoperiod (11.5 h light:12.5 h dark at time of study).
[d]Not examined.

TABLE 5. Leucocrit and hematocrit values for juvenile coho salmon and rainbow trout fingerlings exposed to 0.8 LC50 pulpmill effluent[a] for 24 h after acclimation periods of 1–7 days. Values are expressed as means with SD in parentheses ($n = 12$). P values are based on Student's t-test (variances not assumed to be equal) and represent significance of difference between the corresponding control and effluent-exposed fish. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Acclimation[b] (days)	Leucocrit (%)				Hematocrit (%)			
	Coho		Rainbow		Coho		Rainbow	
	Control	Effluent	Control	Effluent	Control	Effluent	Control	Effluent
0 (stock)	1.30[c] (0.27)		0.92[c] (0.12)		27.88[c] (2.03)		26.79[c] (2.19)	
1	1.12 (0.18)	0.82*** (0.15)	0.80 (0.22)	0.57* (0.15)	28.27 (2.49)	27.00 (2.40)	28.77 (3.96)	28.72 (3.03)
2	1.18 (0.17)	0.70*** (0.14)	0.87 (0.14)	0.62* (0.32)	26.83 (3.83)	27.75 (2.22)	29.92 (3.68)	30.90 (3.18)
3	1.07 (0.13)	0.85*** (0.12)	0.88 (0.14)	0.73 (0.28)	26.58 (3.18)	27.33 (2.67)	29.18 (2.40)	30.73 (5.57)
5	1.08 (0.11)	0.88*** (0.14)	0.93 (0.15)	0.70** (0.09)	28.23 (2.13)	26.63 (1.85)	27.68 (2.19)	29.57 (3.05)
7	1.15 (0.25)	0.82*** (0.13)	0.75 (0.18)	0.70 (0.10)	23.70 (1.49)	26.30* (2.50)	27.50 (3.15)	29.55 (3.27)

[a]BKME sample no. 1.
[b]Number of days following transfer to tanks prior to exposure to effluent.
[c]Pooled value for two groups of 12 fish sampled from the stock tanks at days 0 and 7.

TABLE 6. Leucocrit and hematocrit values for coho salmon and rainbow trout exposed to pulpmill effluent[a] for 4 h after a 2-day acclimation to transfer. Values are expressed as means ± SD value (number of fish in parentheses). P values are based on Student's t-test and represent significance of difference between the respective control sample and the effluent-exposed sample. $^{*}P < 0.05$, $^{**}P < 0.001$.

Effluent concn (portion of 96-h LC50)	Coho salmon		Rainbow trout	
	Leucocrit (%)	Hematocrit (%)	Leucocrit (%)	Hematocrit (%)
stock	1.37±0.37 (15)	27.1±2.5 (15)	0.93±0.22 (15)	26.5±2.9 (15)
0.0 (control)	0.97±0.25 (30)	26.8±2.4 (30)	0.77±0.17 (26)	28.4±2.6 (26)
0.025	1.05±0.25 (15)	27.6±1.5 (15)	0.77±0.20 (15)	29.6±2.1 (15)
0.05	0.97±0.32 (15)	26.5±2.5 (15)	0.78±0.16 (15)	29.9±3.0 (15)
0.1	0.80±0.30* (15)	27.5±2.4 (15)	0.80±0.28 (15)	27.7±2.3 (15)
0.2	0.85±0.23 (15)	26.6±2.4 (15)	0.80±0.22 (15)	30.1±2.1 (15)
0.4	0.90±0.18 (15)	27.2±2.2 (15)	0.68±0.42 (14)	27.7±3.2 (15)
0.6	1.13±0.40 (14)	30.6±2.7** (15)	0.80±0.16 (13)	30.5±2.2* (13)
1.0	1.07±0.30 (15)	30.6±2.6** (15)	0.85±0.20 (14)	29.5±2.6 (14)

[a]BKME sample no. 1.

leucocrit values for either species did not differ from those of controls. Mean hematocrit values for coho were slightly decreased from control values for all groups exposed to effluent for 24 h, but differences were significant ($P < 0.05$) only for those exposed to 0.4 LC50. Similarly, mean hematocrit measurements for rainbow were consistently decreased from control values, with significant depressions at 0.1, 0.4, 1.0 LC50 and both concentrations of treated effluent (Table 7).

TABLE 7. Leucocrit and hematocrit values for coho salmon and rainbow trout exposed to pulpmill effluent[a] for 24 h after a 2-day acclimation to transfer. Values are expressed as means ± SD (number of fish in parentheses). All P values are based on Student's t-test and represent significance of difference between the respective control sample and each effluent-exposed sample. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

Effluent concn (portion of 96-h LC50)	Coho salmon		Rainbow trout	
	Leucocrit (%)	Hematocrit (%)	Leucocrit (%)	Hematocrit (%)
stock	1.29±0.25 (14)	27.5±2.8 (15)	0.96±0.23 (15)	31.0±3.0 (14)
0.0 (control)	1.10±0.26 (24)	27.1±3.6 (24)	0.83±0.18 (20)	31.7±3.3 (20)
0.1	1.09±0.09 (13)	25.7±1.3 (13)	0.75±0.12 (12)	28.8±1.4** (12)
0.2	1.12±0.29 (12)	25.4±1.6 (12)	0.69±0.25 (12)	30.5±2.4 (12)
0.4	0.86±0.09*** (12)	25.1±0.9* (12)	0.71±0.16* (10)	28.7±3.3* (10)
0.6	0.77±0.17*** (12)	26.8±2.4 (12)	0.61±0.19** (12)	29.8±3.2 (12)
0.8	0.84±0.15*** (12)	26.6±2.2 (12)	0.60±0.12*** (12)	29.7±2.8 (12)
1.0	0.85±0.21** (12)	26.0±1.9 (12)	0.61±0.14*** (12)	29.0±3.0* (12)
1.0 treated[b] 90% vol/vol	1.00±0.23 (12)	25.7±1.7 (12)	0.74±0.16 (11)	28.5±2.7** (11)
treated[b]	0.98±0.14 (12)	27.5±1.9 (12)	0.80±0.09 (10)	27.3±3.0*** (12)

[a]BKME sample no. 2.
[b]Detoxified by 5-day aerated lagoon treatment under laboratory conditions.

Discussion

Results from the initial study (Table 1) indicate that blood for leucocrit measurements may be left for at least 12 min prior to centrifugation without significant change. The increase in hematocrit values for the same blood samples is consistent with the findings of Soivio et al. (1973) and is due to swelling of the erythrocytes during storage under lowered oxygen tensions (Soivio et al. 1974). The increase in leucocrit values for rainbow trout and coho salmon after 1 h following centrifugation (Table 2) indicates that centrifuged blood should be examined for leucocrit measurements within 1 h and this interval should be standardized. The portion of blood taken from salmonid fish for hematocrit or leucocrit determinations is probably not of major concern.

It is difficult to attribute the differences observed in mean leucocrit values between fish stocks to specific variables, since many factors were uncontrolled or varied simultaneously. However, there is a need to determine normal hematological values under a wide variety of natural or experimental conditions in order to recognize a pathological state. The leucocrit variations between fish stocks are not ascribed to water temperature or the method of capture and blood collection, since these were held near constant. Although sex has been shown to alter hematocrit values in mature trout (Snieszko 1961), it was not considered here, since all fish groups sampled were sexually immature. The developmental stage of the fish examined is thought to have influenced the leucocrit variations observed; leucopenia has been reported previously for coho salmon fry relative to fingerling or later stages (Katz 1949; McLeay 1975b). The extent to which other variables (degree of crowding, prior disturbances, diet, fish species, size, and strain) affect leucocrit values requires further (controlled) studies.

The decreased WBC-T counts noted for both species due to 96-h stress (high-temperature and crowded conditions) are consistent (although less pronounced) with previous results for yearling coho salmon stressed in the same manner (McLeay 1975a). Reductions in numbers of circulating leucocytes (predominantly small lymphocytes) and thrombocytes appear to typify these species' hematological responses to increased levels of circulating pituitary ACTH and corticosteroid stress hormones, or to acute exposure to stressful conditions (Weinreb 1958; McLeay 1970, 1973b, c). The strong positive correlation of leucocrit values with WBC-T counts in this

study suggests that this simple, rapid method provides a reliable estimate of these counts. The marked depression of leucocrit values of rainbow trout within 6 h indicates that this response is rapidly elicited. Marked decreases in WBC-T counts of juvenile coho salmon within 6–12 h due to stress including BKME exposure were seen previously (McLeay et al. 1972; McLeay 1973a, 1975a).

The elevation of hematocrit values for both subpopulations of rainbow trout exposed for 6–96 h to crowded high-temperature conditions was seen previously for this species during exposure to hypoxic conditions (Swift and Lloyd 1974). This hemoconcentration is believed attributable to a stress-mediated release of erythrocytes from storage organs, or to diuresis. Similarly, erythrocyte counts for coho salmon held under crowded high-temperature conditions for up to 48 h were elevated (McLeay 1975a), although, as with hematocrit for this species in the present study, values did not differ from those of stock fish after 96 h.

Photoperiod extremes can alter the numbers of circulating blood cell types in salmonid fish. McLeay (1970) determined that small lymphocyte and thrombocyte counts were decreased from control (12 h L:12 h D) values in underyearling coho salmon held under conditions of total darkness or flashing lights for up to 20 days, whereas conditions of constant light did not alter differential counts during this period. These hematological changes were correlated with increased activity of the pituitary–interrenal stress axis. Seasonal decreases in the numbers of circulating small lymphocytes and thrombocytes during the winter have been reported for a number of fish species (Schlicher 1927; Gardner and Yevich 1969; McLeay 1975b); however, these changes have been attributed to the stress of cold water temperatures rather than photoperiod effects.

Since in the present study the dissimilar photoperiods under which rainbow trout were reared did not alter any hematological variables measured for the stock or stressed groups, comparable WBC-T or leucocrit responses to acute stress could be anticipated throughout the year for this species regardless of seasonal fluctuations in photoperiod (provided other fish-rearing conditions, including water temperature and developmental stage, were comparable). This consideration is important if a variable such as leucocrit is to prove useful for routine assessments of the health of hatchery stocks or as a standardized procedure for assessing stressful concentrations of pollutants.

The depressions in leucocrit values found for both species at 2–8 days following transfer from stock tanks to freshwater aquaria (controls; Tables 5–7) are attributed to transfer stress. In a previous study where yearling coho salmon were transferred to stream compartments containing freshwater, erythrocyte counts were unaffected by transfer; however, WBC-T counts were depressed from stock values during the 1st day but recovered within 2–4 days and exceeded stock values when acclimations were 7 days or longer (McLeay 1975a). The lack of a similar recovery of leucocrit values for coho salmon or rainbow trout in the present acclimation-effect studies might be due to differences in testing chambers or fish ages.

Acute exposure to sublethal concentrations of kraft pulpmill effluent has been shown repeatedly to depress WBC-T counts in juvenile coho salmon (McLeay et al. 1972; McLeay 1973a, 1975a). Similarly, short-term exposures of this species to other toxicants, including dehydroabietic acid (one of the major toxic constituents in kraft whole mill effluent), at concentrations which didn't alter erythrocyte counts or hematocrit values appreciably, caused marked decreases in WBC-T counts (Bills and Hunn 1976; Iwama et al. 1976). These decreases in WBC-T counts and the present depressions of leucocrit values due to pulpmill effluent exposure are consistent with the stress-mediated hematological responses noted previously (Table 4). The less consistent leucocrit response of rainbow trout to pulpmill effluent relative to coho salmon exposed to the same concentration (Table 5) might reflect differences due to species, developmental stage, or condition of fish stocks prior to these tests.

The significant depressions in leucocrit values in coho or rainbow exposed to a range of BKME concentrations for 24 h after a 2-day acclimation to transfer (Table 7) are in agreement with the observed decreases in WBC-T counts of juvenile coho salmon held in BKME for 24 h after a 2-wk acclimation period (McLeay 1975a; McLeay and Howard 1977). The threshold effect concentrations for WBC-T counts of 0.1–0.2 LC50 for these previous studies are similar to the median effective concentrations of 0.28 LC50 (coho) and 0.16 LC50 (rainbow) found here for leucocrit. The absence of significant changes in leucocrit values for coho salmon or rainbow trout exposed to detoxified effluent for 24 h indicates that this response is due to the toxic constituents rather than the color or inorganic constituents of the effluent. The slight but inconsistent decreases in hematocrit values for fish exposed to pulpmill effluents for 24 h are compatible with the decreases in erythrocyte counts noted previously in

coho salmon exposed to high sublethal concentrations of BKME for the same duration (McLeay 1975a; McLeay and Howard 1977). Since treated wastes also evoked this response in rainbow trout (Table 7), it may be due to effluent characteristics other than toxicity.

The absence of a leucocrit response to BKME within 4-h exposure (Table 6) implies that either a longer exposure to effluent is required before the toxic constituents elicit a stress response, or a longer test duration is necessary before the decrease in circulating leucocytes and/or thrombocytes is manifested. McLeay 1977 has shown that plasma glucose levels in salmonid fish are not elevated by BKME exposures of < 3 h, although a hyperglycemic response is elicited by other stressors within 0.5 h. Thus, the lack of a leucocrit response to BKME within 4 h is thought due, at least in part, to the requirement for a finite exposure period prior to the initiation of a stress response in these fish, and in part to the nature of the manifestation being measured.

Practical Applications

Measurement of leucocrit values is suggested as a simple test method for rapid assessments of changes in the number of leucocytes and thrombocytes in fish blood. In estimating WBC-T counts, this technique offers advantages similar to the hematocrit method for rapid approximations of the numbers of circulating erythrocytes and is amenable to routine fish culture or field studies as an aid to hematological diagnoses of fish condition. Since the buffy layer is a narrow band (sometimes asymmetrical), a small error in measurement may influence results considerably. However, this disadvantage is offset by the considerably greater blood volume (and thus more representative sample) used for leucocrit than for WBC-T counts. In cases where considerable hemolysis of centrifuged blood is evident, with concomitant contamination of the buffy layer, the leucocrit technique should not be employed.

The present findings support the suggestion of Belova (1966) that a decrease in numbers of circulating lymphocytes is a more reliable indicator of unfavorable environmental conditions and stress than hematocrit, erythrocyte counts, or any other hematological parameters, and that of Hickey (1976) that changes in leucocyte counts may well represent the most definitive test in determining physiological responses of fish to toxic substances. The role of leucocyte cell types in the immunological defence mechanisms in fish and the effects of environmental stressors on outbreaks of disease are becoming increasingly clear (Snieszko 1974; Corbel 1975; Ellis et al. 1976; Etlinger et al. 1976). Measurement of leucocrit values might prove useful in the early detection of incipient disease conditions and in the selection of healthy stock for breeding purposes.

At least for salmonid fishes, leucocrit determinations appear to provide a helpful means for assessing concentrations of pulpmill effluent that are acutely stressful. A 24-h exposure after a 2-day acclimation to transfer results in a consistently sensitive response to this effluent and is presently considered to be a practical test paradigm for measuring stressful concentrations of effluent under controlled environmental conditions. Knowledge as to the effects of transient exposures and recoveries in freshwater on the time responses of leucocrit values might suggest useful procedural variations and would help indicate the relevance of this response when applied to field situations. Additionally, studies are required to assess the applicability of this response to other species (including higher vertebrates) and toxicants.

In juvenile coho salmon receiving chronic exposure to pulpmill effluent, WBC-T counts are not altered from control values (McLeay 1973a; McLeay and Brown 1974). This recovery to normal values with prolonged exposures is considered typical of Selye's (1950) stage of resistance to stress for higher vertebrates. Leucocrit values are probably not a useful indicator of chronic stress conditions in fish.

Acknowledgments

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Colonization and Decomposition of Fish Bone Material in Natural and Synthetic Aqueous Solutions

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SMITH, E. A. H., C. I. MAYFIELD AND P. T. S. WONG. 1977. Colonization and decomposition of fish bone material in natural and synthetic aqueous solutions. J. Fish. Res. Board Can. 34: 2176–2184.

Partial dissolution of the inorganic (apatite) fraction of flesh-free fish (*Perca sp.*) cheek-bone material, added to P-free (<0.001 $\mu g \cdot ml^{-1}$ total P) nutrient media as bone chips with a particle-size distribution of <250 μm, supported considerable populations of algae and bacteria at slightly alkaline pH levels and relatively low slurry densities (1–1000 $\mu g \cdot ml^{-1}$). Bacteria and algae rapidly colonized the apatitic bone material and produced extracellular material in response to growth on bone surfaces. Acidic filtrates from bacterial cultures, aerobically or anaerobically cultivated in apatite media, readily solubilized bone material as indicated by increases in soluble ortho-PO_4^{3-} concentrations after short exposure periods. Water extraction of bone apatite crystals showed that fish bones are sparingly soluble in lake water. Microbially mediated dissolution of bone material by acid end-products of bacterial metabolism would therefore greatly increase the rate of bone dissolution in nature. Regeneration of allochthonous-P from fish-bone dissolution may contribute significantly to the P-cycle of many aquatic ecosystems.

Key words: biologic apatite, calcium phosphate, fish bone carbonate hydroxyapatite, dahllite

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La fraction inorganique (apatite) partiellement dissoute de matériel d'opercule de poisson (*Perca* sp.) libre de chair, ajoutée à un milieu de culture libre de P (<0.001 $\mu g \cdot ml^{-1}$ de P total) en fragments dont la grosseur est répartie sur une gamme de <250 μm, peut alimenter des populations considérables d'algues et de bactéries à des niveaux de pH légèrement alcalins et à des densités de pâte relativement basses (1–1000 $\mu g \cdot ml^{-1}$). Les bactéries et les algues colonisent rapidement le matériel osseux apatitique et produisent un matériel extracellulaire en réponse à la croissance sur la surface osseuse. Les filtrats de caractère acidique provenant des cultures bactériennes aérobies ou anaérobies en milieux d'apatite dissolvent facilement le matériel osseux comme l'indiquent les augmentations de teneurs en ortho-PO_4^{3-} soluble après de courtes expositions. L'extraction à l'eau des cristaux d'apatite des os démontre que les os de poisson sont peu solubles dans de l'eau de lac. Une dissolution, facilitée par action microbienne, du matériel osseux par les produits finals acides du métabolisme bactérien augmenterait donc considérablement la vitesse de dissolution des os dans la nature. Il est possible que la régénération du P allochtone résultant de la dissolution des os de poisson contribue de façon significative au cycle du phosphore de plusieurs écosystèmes aquatiques.

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FOR many years, apatites have been actively investigated due to the ubiquitous distribution of this mineral in nature. Geologically, apatite (hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), fluorapatite ($Ca_{10}(PO_4)_6F_2$), and chlorapatite ($Ca_{10}(PO_4)_6Cl_2$)) can be found associated with igneous, metamorphic, sedimentary, and even lunar rocks (Albee and Chodos 1970; McConnell 1973).

Biologically, apatite is important as the major component of teeth and bones of vertebrates. Biological apatites differ from other forms in that substantial quantities of H_2O and CO_3^{2-} are

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found within the crystal lattice structure of these mineralized tissues (McConnell 1973). Biological apatites can be divided into two major categories based on fluoride concentration: dahllite (carbonate hydroxyapatite), where the fluoride concentration is <1%, and francolite (carbonate fluorapatite), where the fluoride concentration is >1% (McConnell 1973).

Ecologically, apatite has been shown to constitute a large fraction of the P that is entering lake environments, particulary from shoreline erosion (Burns et al. 1976; Williams et al. 1976). There is, however, little information describing the contribution of other sparingly soluble calcium phosphates such as fish bones to the P-cycle of aquatic environments. The present investigation was conducted to examine the microbial colonization of fish bone material and examine the factors affecting the biological availability the ortho-PO_4^{3-} associated with this form of biological apatite in water and sediment.

Materials and Methods

Bone Material

Flesh-free cheek bones from fish (*Perca sp.*), separated into organic pulp (brown) and inorganic crystal (white) fractions, were ground with mortar and pestle, passed through sieves to obtain particle sizes of <250 μm, dried for 12 h at 100°C, and stored in sterile vials.

Algal Culture Studies

Each bone fraction was added to acid-washed 150-ml flasks containing 50-ml quantities of P-free (<0.001 $\mu g \cdot ml^{-1}$ total P) Bristol's (PfB) medium (Nichols and Bold 1965) at pH 7.5 as the sole source of ortho-PO_4^{3-} to yield 1.0, 10, 100, and 1000 $\mu g \cdot ml^{-1}$ of bone material. P-free nutrient medium was maintained as a control. The flasks were inoculated with 0.1 ml of washed, nonaxenic cells of *Scenedesmus quadricauda* (77; Starr 1964) and *Ankistrodesmus braunii* (ATCC 2744), which had been cultivated under P-limiting conditions to yield approximately 20 cells·ml^{-1}. The cultures were incubated for 10 days on a rotary shaker (100 rpm) at 20°C under conditions of 18 h of light (3800 lx) and 6 h of darkness. The number of algae in the media was determined at 24-h intervals using a Petroff-Hauser counting chamber and a phase-contrast microscope.

Bacterial Culture Studies

Bone apatite crystals were added to 250-ml flasks containing 100-ml quantities of P-free (<0.001 $\mu g \cdot ml^{-1}$ total P) heterotrophic medium (Liu et al. 1973) at pH 7.2 to yield 1.0, 10, 100, and 1000 $\mu g \cdot ml^{-1}$ of bone material. The flasks were inoculated with 0.1 ml of washed cells of an unidentified bacterial population cultivated under P-limiting conditions to yield approximately 100 cells·ml^{-1}. The flasks were incubated at 20°C in the dark on a rotary shaker (100 rpm) and the number of microorganisms per millilitre was determined at 24-h intervals during a 7-day incubation as outlined above. Flasks inoculated with 0.1 ml of a 1:1000 dilution of Lake Ontario sediment were aerobically incubated or evacuated and back-filled with N_2 for comparative purposes.

Bone crystals were added to 250-ml flasks containing 100-ml quantities of epilimnetic water from Lake Ontario (pH 7.8) to yield 100 and 1000 $\mu g \cdot ml^{-1}$ of bone apatite. Replicate flasks were inoculated with 1 ml of a 1:10 dilution of Lake Ontario sediment. Apatite bone crystals were examined by scanning electron microscopy at 24-h intervals.

Water Extraction of Bone Material

A 100-mg quantity of bone apatite, previously washed with distilled water (pH 8.0), was added to a 50-ml, acid-washed flask containing 25 ml of filtered (0.45 μm) epilimnetic water from Lake Ontario (pH 7.8) with endogenous levels of 38.1 and 0.03 $\mu g \cdot ml^{-1}$ of soluble Ca^{2+} and ortho-PO_4^{3-}, respectively (Anon. 1974). After 1 min the contents of the flask were filtered through a 0.45 μm membrane filter and the filtrate was retained for ortho-PO_4^{3-} analysis. Ten successive 25-ml quantities of filtered lake water were then passed through the filter under suction (5 atm) and the levels of ortho-PO_4^{3-} in the filtrates were determined. The process was repeated using the filtrate of a 10-day culture aerobically cultivated in P-free heterotrophic medium containing 1000 $\mu g \cdot ml^{-1}$ of bone apatite (pH 5.4).

At 24-h intervals the contents of selected aerobically or anaerobically incubated flasks containing synthetic apatite-nutrient media were filtered through 0.45 μm filters and the concentrations of soluble ortho-PO_4^{3-} in the filtrates following a 1-min exposure to 100 mg of washed bone apatite crystals were also determined.

Scanning Electron Microscopy

To examine colonization of bone crystals, 2.5-ml samples from algal and aerobic heterotrophic cultures were collected at 24-h intervals on Millipore filters (0.45 μm) by gentle aspiration and immediately immersed in 2.5% glutaraldehyde buffered with 1.5 M cacodylate buffer, pH 7.4, for 1 h. After thorough washing the cells were postfixed in 1.5% buffered osmium tetroxide for 30 min. The preparations were then rinsed in buffer, washed 3 times in glass-distilled deionized water, and dehydrated through a graded ethanol series. When the material was thoroughly infiltrated with 100% ethanol, the alcohol was replaced by 100% amyl acetate by passing the material through a graded series of mixtures of the two liquids. The specimens were quickly transferred to a precooled, high-pressure, specimen-drying chamber (BOMAR Critical Point Apparatus, SPC 900/EX) and dried using carbon dioxide as the transitional fluid (Anderson 1951). The specimens, mounted on aluminum stubs, were coated with a layer of gold to a thickness of 20

nm and examined by an AMR 1000 scanning electron microscope.

Results

The inorganic (apatite) and organic fractions of perch cheek-bone supported the growth of algae and bacteria in P-free synthetic nutrient media (Table 1). In each case, as the amount of bone material was increased the amounts of ortho-PO_4^{3-} released into solution increased as indicated by increases in microbial biomass. After a 10-day incubation at 20°C, PfB media (pH 7.5) containing 1.0, 10, 100, and 1000 $\mu g \cdot ml^{-1}$ of bone apatite inoculated with nonaxenic *S. quadricauda* and *A. braunii* cells to yield approximately 20 cells·ml^{-1} gave counts of 960, 3440, 5840, and 20,800 cells·ml^{-1}. No significant growth was observed in the control medium during the experiment. The organic fraction of the bone also contained a considerable amount of available-P, as indicated by the development of substantial numbers of algae (Table 1).

Similarly, sufficient ortho-PO_4^{3-} was also released by partial dissolution of the bone material in P-free heterotrophic media at a pH of 7.2 to support large populations of aerobic and anaerobic microorganisms. A concentration of 100 $\mu g \cdot ml^{-1}$ of bone apatite caused more than a 200-fold increase in the number of aerobes cultivated during a 7-day incubation at 20°C (Table 1). The organic fraction of bone seemed to support the growth of fewer numbers of microorganisms than the apatite fraction. It is noteworthy that the numbers of bacteria and algae as determined by direct count represent underestimates of the actual increases in cell numbers because the microbial growth on the surfaces of the bone apatite crystals which occurred during the incubations was not determined.

Scanning electron microscopic examination of bone apatite selected from algal cultures after various incubation periods revealed that *S. quadricauda* and *A. braunii* colonized the bone material and produced large amounts of extracellular fibrous material as a mechanism of attachment to the surfaces of bone fragments (Fig. 1). Many of the *S. quadricauda* cells displayed a unicellular, as opposed to colony, morphology (Fig. 2). Smaller bone apatite crystals were found attached to the surfaces of *S. quadricauda* (Fig. 3) and *A. braunii* cells (Fig. 4) and were also clustered in various locations along the strands of extracellular material (Fig. 3). The greater portion of the extracellular fibers seemed to be associated with the *S. quadricauda* cells which displayed the unicellular morphology (Fig. 2).

Bacteria rapidly colonized the surfaces of bone apatite crystals in the presence of algae (Fig. 4) and in algae-free cultures (Fig. 5). The bacterial population colonizing the bone material was composed predominantly of bacillary forms (Fig. 6), and bone fragments typically showed coatings of bacteria, often several cells thick (Fig. 7). After a 7-day incubation, massive amounts of extracellular fibrous material, produced in response to growth on bone fragments, formed intercellular polymeric bridges and attached to surfaces of the bone material (Fig. 8). Similar growth of microorganisms on bone apatite was observed in epilimnetic Lake Ontario water (Fig. 9) and in lake water inoculated with a sediment slurry (Fig. 10).

Exposure of bone crystals to culture filtrates from heterotrophic microorganisms cultivated in apatite media showed the instability of this form of biological apatite under acidic conditions. The pH levels of aerobic and anaerobic cultures containing 1000 $\mu g \cdot ml^{-1}$ of bone material decreased from 7.2 to 5.6 and 5.2, respectively, during a 7-day incubation at 20°C (Table 2). The amounts of ortho-PO_4^{3-} ions released from 100 mg of bone material during a 1-min exposure to the

TABLE 1. Effect of bone material on numbers of algae and bacteria in synthetic P-free nutrient media.

Concn bone material ($\mu g \cdot ml^{-1}$)	Algae[a]		Bacteria[a]	
	Inorganic bone fraction	Organic bone fraction	Inorganic bone fraction	Organic bone fraction
0	73	85	550	420
1	960	560	7,600	5,100
10	3,440	2,100	54,000	27,500
100	5,840	7,680	111,000	77,000
1,000	20,800	17,900	340,000	170,000

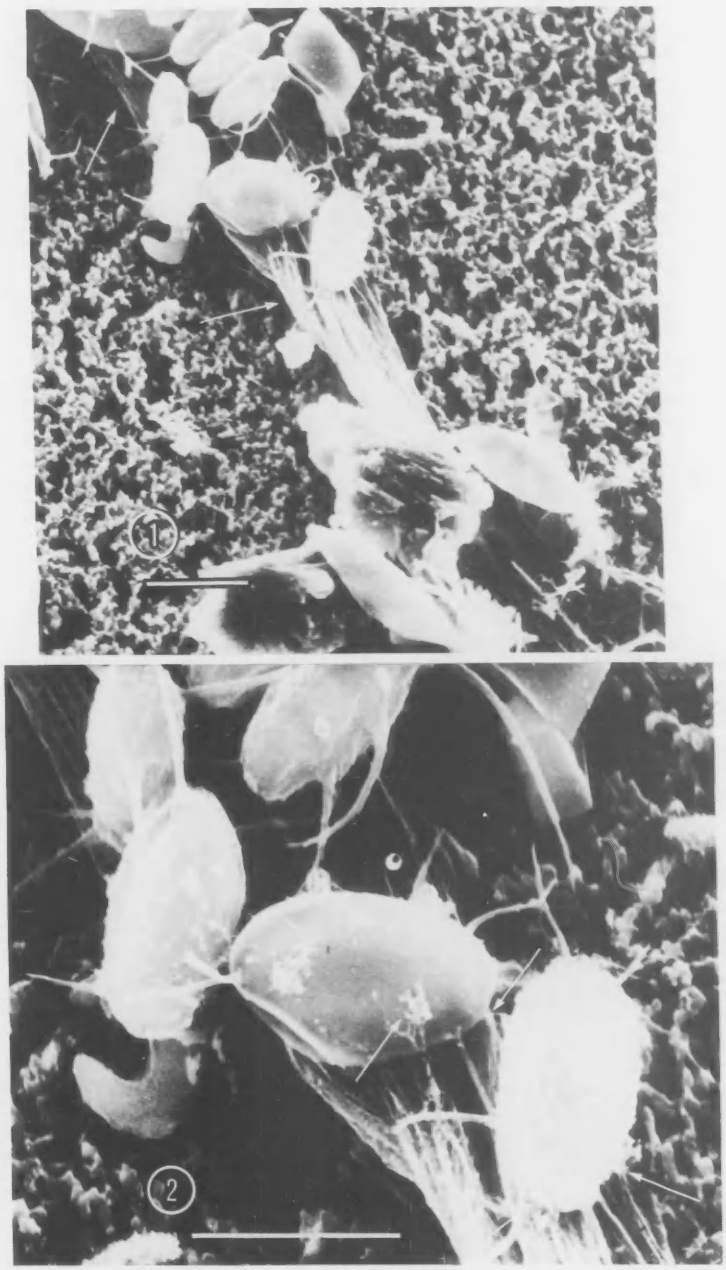
[a]Numbers of algae per millilitre (*A. braunii* and *S. quadricauda*) and bacteria (unidentified) as determined by direct microscopic count after incubation at 20°C (algae — 10 days, bacteria — 7 days).

TABLE 2. Effect of culture pH on solubilization of bone apatite.

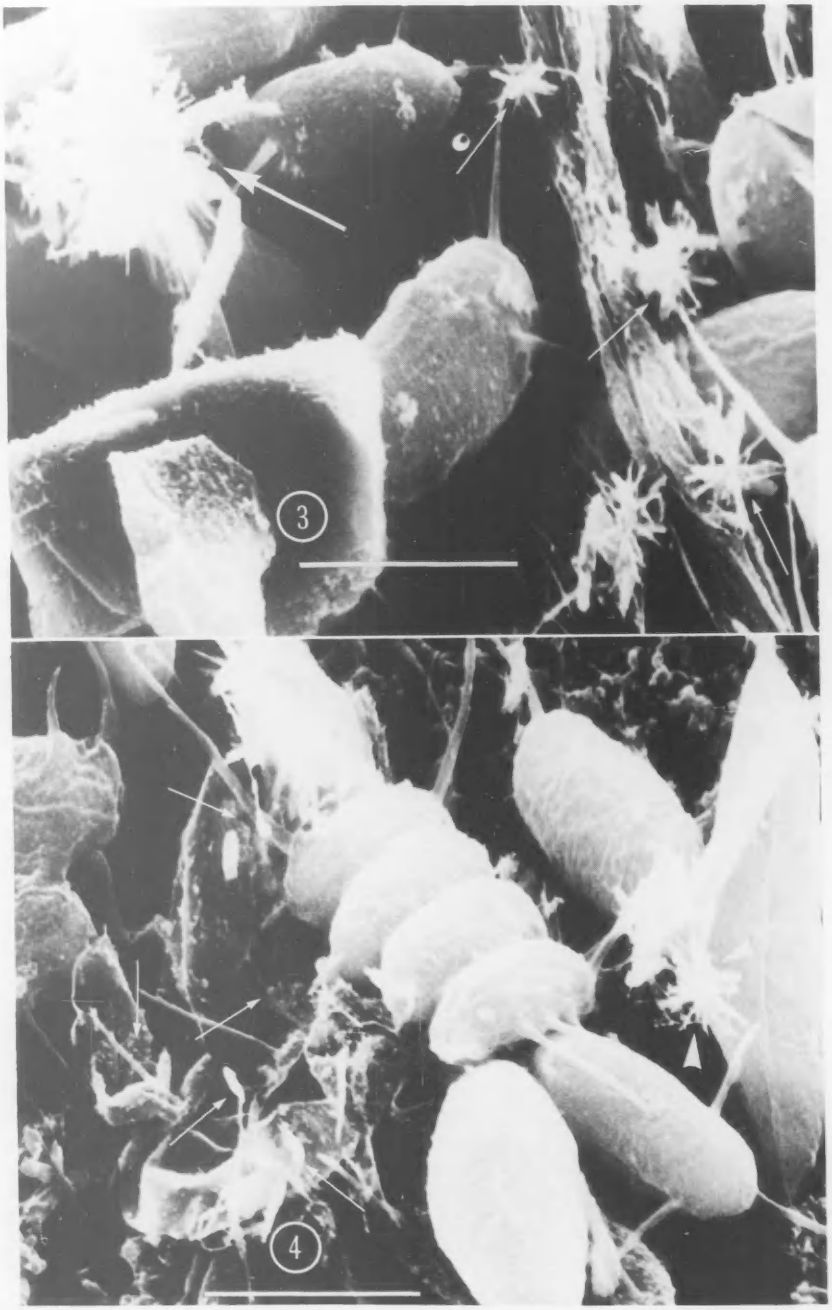
Time[a] (days)	Culture pH		Soluble ortho-PO_4^{3-} released ($\mu g \cdot ml^{-1}$)[b]	
	Aerobic	Anaerobic	Aerobic	Anaerobic
1	7.2	7.2	0.800	0.825
2	7.0	7.1	0.875	0.900
3	6.9	6.8	1.100	1.200
4	6.7	6.2	1.225	1.325
5	6.3	5.9	1.650	1.725
6	5.9	5.6	1.850	1.800
7	5.6	5.2	1.900	2.100

[a]Cultures of heterotrophic microorganisms in apatite-containing medium incubated for various times under aerobic and anaerobic conditions.

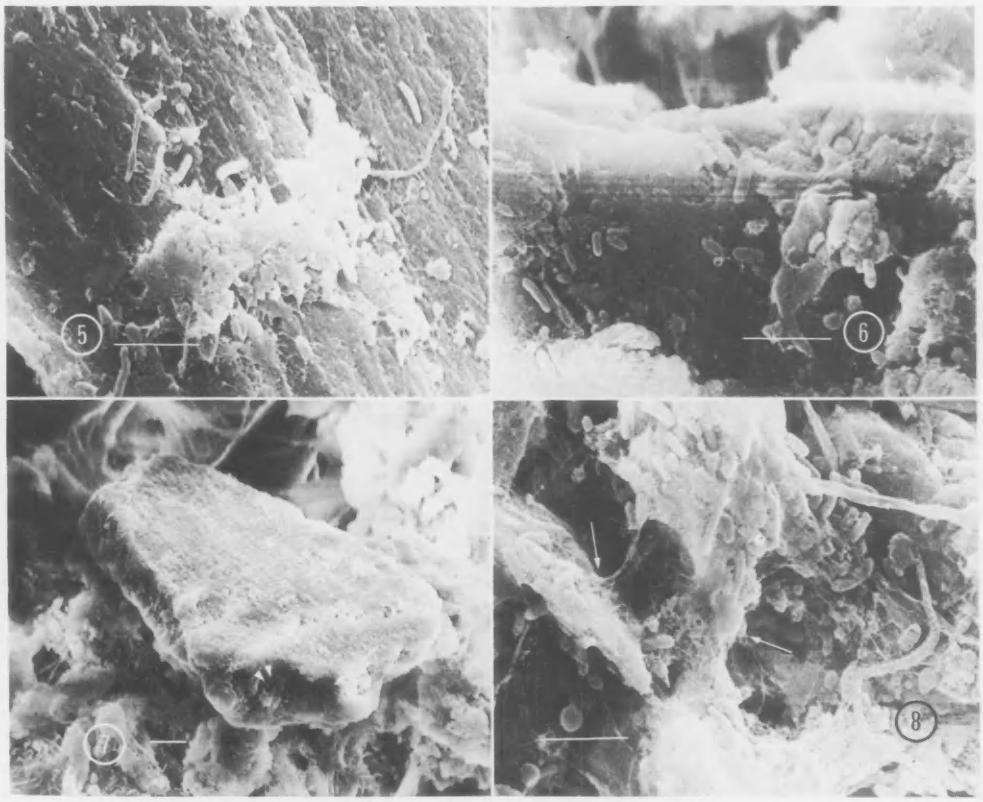
[b]100 mg of bone apatite crystals washed in distilled water (pH 8.0) were exposed for 1 min to 25-ml quantities of culture filtrates.



FIGURES 1 AND 2. Scanning electron micrographs of *Scenedesmus quadricauda* and *Ankistrodesmus braunii* cultivated for 10 days in P-free Bristol's medium with bone apatite crystals added as the sole source of ortho-PO_4^{3-} to yield 1000 $\mu g \cdot ml^{-1}$. FIG. 1. Large amounts of extracellular fibrous material (arrows) as a mechanism of attachment to bone fragments. FIG. 2. The extracellular fibers appeared to be produced at various locations (arrows) on the outer surfaces of the algal cells. Scale = 15 μm.



FIGURES 3 AND 4. Same culture as shown in Fig. 1 and 2. FIG. 3. Clusters of apatite crystals appeared to be attached to the surfaces of algal cells (large arrow) as well as to extracellular fibers (small arrows). FIG. 4. *Scenedesmus quadricauda* cells appeared in groups of four, in pairs and as unicells. Bacterial colonization of dead algae (small arrows) and a cluster of bone apatite crystals attached to an *A. braunii* cell (arrow head) are also depicted. Scale = 15 μm.



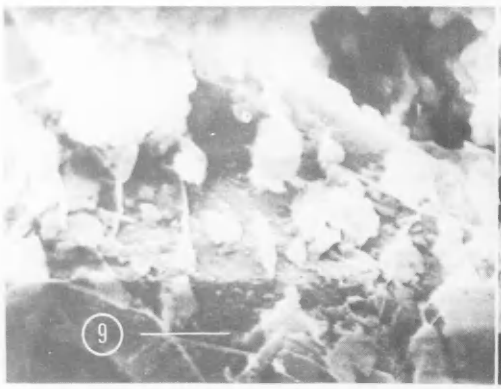
FIGURES 5–8. Scanning electron micrographs of bone apatite crystals after different incubation periods in P-free media containing an unidentified population of heterotrophic microorganisms cultivated at 20°C under aerobic conditions. FIG. 5. Bacterial colonization of bone material was evident after 48 h of incubation. FIG. 6. Microbial growth on bone fragments increased and after 4 days it was evident that bacillary forms were primarily involved in the colonization of bone material. FIG. 7. After a 7-day incubation, the surfaces of bone crystals were coated with layers of bacteria, often several layers thick. In the small clear area of the bone fragment, cocci (arrow heads) and bacilli (arrow) appear to have colonized the bone material. FIG. 8. The microbial population produced massive amounts of extracellular material which extended intercellularly and also enveloped bone crystals (arrows). Scale = 5 μm.

culture filtrates increased as the pH levels decreased. The concentration of ortho-PO_4^{3-} ions in aerobe filtrates with pH levels of 7.0 and 5.9 were 0.875 and 1.850 $\mu g \cdot ml^{-1}$, respectively (Table 2).

Fish bone material is partially soluble in lake water (Table 3). Ten successive extractions of 100 mg of bone crystals with lake water (pH 7.8) containing endogenous concentrations of soluble ortho-PO_4^{3-} and Ca^{2+} of 0.03 and 38.1 $\mu g \cdot ml^{-1}$, respectively, released a cumulative amount of ortho-PO_4^{3-} of 1250 μg. Similarly, extraction of the same amount of bone material using a culture filtrate with a pH level of 5.4 released a total of 2900 μg of ortho-PO_4^{3-}. In each case the levels of ortho-PO_4^{3-} released from the bone apatite decreased with each successive extraction.

Discussion

The cheek bone of the perch, a representative fish of freshwater ecosystems, was used in these experiments as a source of apatite to examine the colonization and decomposition of bone material. Microorganisms rapidly invade the tissues of dead fishes and microbial decomposition of this organic debris will contribute numerous elements to the nutrient cycling of aquatic environments. However, the fact that fish bones do not accumulate in many situations indicates that the decomposi-



FIGURES 9 AND 10. FIG. 9. Growth of microorganisms on bone apatite incubated for 7 days at 20°C in epilimnetic Lake Ontario water. Scale = 5 μm. FIG. 10. Attachment of bacteria and organic debris to bone apatite (arrows) incubated for 7 days at 20°C in epilimnetic Lake Ontario water inoculated with 0.1 ml of a 1:1000 dilution of Lake Ontario sediment. Scale = 5 μm.

TABLE 3. Water extraction of bone apatite crystals.

Extraction number[a]	Soluble ortho-PO_4^{3-} released ($\mu g \cdot ml^{-1}$)	
	Lake water (pH 7.8)	Culture filtrate[b] (pH 5.4)
Control	0.030	0.001
1	1.950	3.900
2	0.970	2.100
3	0.700	1.300
4	0.550	0.980
5	0.400	0.820
6	0.325	0.750
7	0.200	0.600
8	0.125	0.550
9	0.075	0.400
10	0.050	0.325
Total	5.045	11.625

[a]100 mg of bone apatite crystals were exposed for 1 min to 25-ml quantities of lake water or culture filtrate.

[b]10-day culture aerobically cultivated in P-free heterotrophic medium containing 100 $\mu g \cdot ml^{-1}$ of bone apatite.

tion processes are not restricted to the fleshy material.

Although apatites characteristically display low solubilities in aqueous solutions at alkaline pH levels (Brown 1966), sufficient ortho-PO_4^{3-} was released by the partial dissolution of bone apatite crystals at a pH level of 7.5 in a P-free nutrient medium to support substantial populations of algae at relatively low slurry densities. Although bone material may not occur as small crystals in the environment, this study served to indicate the availability of ortho-PO_4^{3-} associated with this form of sparingly soluble calcium phosphate.

It is assumed that the major portion of fish bone decomposition occurs either at the sediment–water interface or near the surface of sediment. The water-extraction technique used here suggested that a certain amount of fish bone material will be solubilized by water erosion. These findings also suggest that this process may be slow and that microbial dissolution of the bone material would be the primary method of degradation. It has been shown that although Lakes Erie and Ontario water are undersaturated with respect to hydroxyapatite (Sutherland et al. 1966), the interstitial water of several Lake Ontario sediment samples was exactly saturated. Thus, under natural conditions, physical dissolution in interstitial waters and current action likely account for less fish bone solubilization that that produced by action of microorganisms.

Exposure of the apatite fraction of fish bone material to bacterial culture filtrates verified the instability of apatite in acidic aqueous solutions. These results suggest that acid end-products of microbial metabolism would be the primary mechanism of degradation of the mineralized tissue in situ. These findings are in agreement with those of other workers who found that several soil microorganisms including fungi, actinomycetes, and bacteria were capable of dissolving "insoluble" phosphatic minerals by formation of acid end-products of carbohydrate metabolism (Sperber 1958a, b; Johnston 1959). These workers found that some of the acid end-products of metabolism that have been shown to be most efficient in chelation and solubilization of in-

organic phosphates are lactic, glycolic, citric, succinic, oxalic, pyruvic, and furoic acids. Muromtsev (1958) has also shown that microorganisms can dissolve calcium phosphates in acid, neutral, and alkaline culture media.

Although Deitz et al. (1964) have postulated the formation of a surface complex ($Ca_2HPO_4(OH)_2$) to account for the relative insolubility of hydroxyapatite, if such a complex forms on the surface of crystals in the environment, it does not appear to affect the microbially mediated dissolution of fish bones.

Our results suggest that a number of factors will play a role in the microbial solubilization of fish bones in nature. The dissolution process will occur more rapidly as temperature increases and will occur under aerobic and anaerobic conditions and will be dependent to a large extent on the amount of available C for production of acid end-products of microbial metabolism. The labile components of organic detritus may supply C for heterotrophic dissolution of bone material. Other workers have reported that the factor determining the distribution of phosphate-solubilizing bacteria in lake bottom sediments was the organic matter content and the degree of its mineralization; they also found that there was no relation between the oxygen content in the water and the number of phosphate-solubilizing bacteria developing in it (Niewolak 1971).

Under natural conditions, the P of fish bones may be restricted to the sediments. However, it may be possible for some of the ortho-PO_4^{3-} to become available for algal growth by the action of water currents or during seasonal overturns. Although sufficient ortho-PO_4^{3-} was released by partial dissolution of fish bone material to support a substantial algal population in a synthetic P-free nutrient medium, it is difficult to determine whether fish bone dissolution will contribute enough P to primary production to be environmentally important.

Large populations of bacteria and algae verified that the organic fraction of fish bone also contains available ortho-PO_4^{3-} for microorganisms and it was found that bacteria and algae produced large quantities of extracellular material as a mechanism for attachment to bone apatite crystals. Adsorption of microorganisms onto bone apatite may be analagous to the adsorption of oral microorganisms onto the hydroxyapatite surfaces of tooth material (Ericson et al. 1975). Growth of microorganisms on the surface of apatite crystals in nature may influence their spatial and temporal distribution.

There are little or no data available determining the number of fish deaths each year or the amounts of fish flesh and bone contributed to lake waters and sediments. Thus, it would be difficult to accurately estimate the contribution of fish bones to the P-cycle of aquatic environments. However, if 10% of a fish's weight is bone material, for every 10,000 tons of dead fish, the solubilization of bone tissue (1000 tons) would contribute several hundred tons of soluble ortho-PO_4^{3-} to the P-cycle, the contribution being more significant in highly productive waters. Man's activities have increased the amounts of readily available ortho-PO_4^{3-} in lakes which in turn reduces the significance of other P sources. However, the reduction of anthropogenic P-loadings may increase the importance of the less available forms of P such as those associated with fish bone material.

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Effects of Pseudobranchectomy on Visual Pigment Density and Ocular PO_2 in Atlantic Salmon, *Salmo salar*

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Bilateral pseudobranchectomy (B.P.) of Atlantic salmon, *Salmo salar*, resulted in a progressive loss of visual pigment and a marked decrease in ocular PO_2 near the retina. B.P. fish were dark and blind within a few hours of pseudobranchectomy. Unilateral pseudobranchectomy (U.P.) did not have a similar effect on ocular PO_2 or visual pigment density in either the ipsilateral or the contralateral eye. U.P. fish did not become dark or blind. An interconnection exists between the right and left ophthalmic arteries permitting an intact pseudobranch to supply blood to the contralateral eye whose pseudobranch has been removed. Although intramuscular injection of acetazolamide caused an initial decrease in amount of visual pigment, body darkening, and blindness, these effects were not permanent. Acetazolamide also caused a marked decrease in ocular PO_2 similar to that in B.P. fish. In control fish, dorsal aortic blood and ocular PO_2 values were dependent upon an adequate irrigation of the gills.

Key words: salmonid, *Salmo*, pseudobranch, oxygen tension, eye, visual pigment

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La pseudobranchectomie bilatérale (P.B.) du saumon atlantique, *Salmo salar*, entraîne une perte progressive du pigment visuel et une diminution marquée du PO_2 oculaire près de la rétine. Les poissons ainsi traités deviennent noirs et aveugles en moins de quelques heures après la pseudobranchectomie. La pseudobranchectomie unilatérale (P.U.) n'a pas d'effet significatif sur le PO_2 oculaire ou sur la densité du pigment visuel, ni dans l'œil ipsilatéral ni dans l'œil contralatéral. Les poissons soumis à la P.U. ne deviennent pas noirs ni aveugles. Il y a interconnection entre les artères ophthalmiques droite et gauche, permettant à une pseudobranchie intacte d'irriguer l'œil contralatéral privé de sa pseudobranchie. Une injection intramusculaire d'acétazolamide cause une diminution initiale de la quantité de pigment visuel, le noircissement du corps et la cécité, mais ces effets ne sont pas permanents. L'acétazolamide cause également une diminution marquée du PO_2 oculaire semblable à celle des poissons soumis à la P.B. Chez les témoins, les valeurs relatives au sang de l'aorte dorsale et celles du PO_2 oculaire dépendent d'une irrigation adéquate des branchies.

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OXYGEN partial pressures well above the air saturation value (152 mm Hg) have been recorded in the vitreous humor near the retina of fish eyes (Wittenberg and Wittenberg 1962, 1974; Fairbanks et al. 1969, 1974). Wittenberg and Wittenberg (1974) and Wittenberg and Haedrich (1974) have reviewed the literature concerning the pseudobranch and the oxygen-concentrating system in the choroid rete-choriocapillaris of fishes. The ophthalmic artery, which originates from the pseudobranch, supplies blood to the choroid rete and then to the choriocapillaris (Barnett 1951). Consequently, any disruption of this blood supply, such as occurs with pseudobranch removal, may cause impairment in the visual system. Removal of both pseudobranchs results in body darkening (Parry and Holliday 1960) and blindness, as judged by the behavior of the fish (Denton and Saunders 1972). Because there is an interconnection between the ophthal-

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mic arteries, the present paper will show that unilateral pseudobranchectomy does not produce these effects. Fairbanks et al. (1969, 1974) attribute the drop in oxygen partial pressure (PO_2) near the retina, following pseudobranchectomy, to impairment of the oxygen-concentrating mechanism in the choroid rete of the eye.

Wittenberg and Haedrich (1974) suggest that the pseudobranch (rich in carbonic anhydrase) alters the arterial blood going to the choroid rete, preventing an excessive concentration of carbon dioxide from accumulating at the distal end of the choroid rete during the oxygen-concentrating process. In support of this hypothesis, Maetz (1956) found a marked decrease in the pH of the vitreous humor following injection of acetazolamide (a potent inhibitor of carbonic anhydrase).

In general, teleosts that do not have pseudobranchs either lack or have poorly developed choroid retia with the exception of certain freshwater species (Wittenberg and Haedrich 1974). Most evidence suggests that the pseudobranch and the choroid rete function in consort. In the present study, Atlantic salmon, *Salmo salar*, were subjected to unilateral or bilateral pseudobranchectomy or to acetazolamide injection. The density of visual pigment in retinal extracts and the oxygen tension (PO_2) in the vitreous humor near the retina were determined at specific times following pseudobranchectomy or injection in order to elucidate further the role of the pseudobranch in the function of the teleost eye.

Materials and Methods

Visual Pigment Density

Atlantic salmon parr (mean weight 40 g, mean length 16 cm) were anesthetized with ethyl *m*-aminobenzoate methanesulfonate (MS-222®) and either bilaterally (B.P.) or unilaterally (U.P.) pseudobranchectomized by cautery with a small soldering iron. The fish were then returned to illuminated tanks and held at 10°C in freshwater. A second group of parr was sham operated by cautery of a region adjacent to the pseudobranch and maintained under identical conditions for use as controls. A third group was injected (intramuscular in the caudal region) with 25 μg of acetazolamide and also held at 10°C in illuminated tanks.

At specific times following pseudobranchectomy or acetazolamide injection, individuals were dark adapted for 1 h, anesthetized with MS-222 and the eyes were removed in dim red light (Wratten Series 2). Control fish were treated in a similar manner. Retinas were then removed, placed in 4% potassium aluminum sulfate, and stored at −20°C in lighttight containers. Retinas from the eyes of a U.P. fish were kept separate, whereas those from a B.P. fish were combined. Later, visual pigment extracts were prepared in 2% digitonin following a standard extraction procedure (Beatty 1969). The extract volume was 0.5 ml for all samples.

To determine the density of visual pigment in a sample, the absorbance spectrum of an extract was measured in a microcuvette (1.0 cm path length) from 680 to 340 nm with a Cary 14 recording spectrophotometer. The extract was then exposed to orange light (610 nm, white light passed through a Baird-Atomic interference filter) for 10 min to bleach the visual pigment totally. The absorbance spectrum of the bleached extract was then measured and the maximum absorbance change determined by subtraction from the absorbance spectrum of the unbleached extract. To prevent any visual pigment regeneration, 0.05 ml of 0.2 M neutralized hydroxylamine was added to the extract prior to the initial recording of the absorbance spectrum.

Ocular PO_2 Measurements

Atlantic salmon smolts (mean weight 167 g, mean length 26 cm) were anesthetized (MS-222) and either bilaterally or unilaterally pseudobranchectomized by cautery. Smolts were also sham operated by cautery of a region adjacent to the pseudobranch. Several unoperated smolts were given single injections of acetazolamide (5 μg/fish) through a dorsal aorta cannula implanted by using the procedure of Smith and Bell (1964). The fish were then returned to illuminated tanks and held at 15°C in freshwater.

For measuring ocular PO_2 a fish was lightly anesthetized and placed in a sling, and its gills were irrigated, through a tube placed in the mouth, with aerated freshwater (15°C) containing sufficient MS-222 to cause heavy anesthesia. A 20-ga. hypodermic needle was then used to puncture the cornea and a 6-mm length of PE50 polyethylene tubing was inserted in the hole left by the needle to a depth of 4 mm from the corneal surface by passing it between the lens and the iris. This tube served as a guide for inserting the oxygen microelectrode into the vitreous humor with a micromanipulator. A series of measurements showed that the cornea to retina distance was between 7 and 8 mm. The position of the electrode tip with respect to the surface of the cornea was recorded for every PO_2 measurement. A series of PO_2 measurements was made as the electrode was inserted in 1-mm steps to a point near the retina and during its stepwise removal. Ocular PO_2 measurements were made similarly in the acetazolamide-injected fish at specific times following the injection. Unoperated, uninjected smolts were used for determining normal ocular PO_2 levels.

Our oxygen microelectrodes (Maes 1976) were of the Clark type, consisting of a glass-insulated platinum cathode (20 μm) surrounded by an epoxy-insulated, chlorided silver anode. The tip of the electrode was covered by a layer of collodion containing 2 M KCl surrounded by a thin DPX membrane. The 95% response time was 2–5 s. Prior to and after every experiment, the microelectrode was

calibrated in O_2-saturated water, air-saturated water, and deoxygenated water at the experimental temperature (15°C). The amplified signal from the microelectrode was displayed on a cathode-ray oscilloscope.

Dorsal Aorta PO_2 Measurements

To determine whether irrigation of the gills of anesthetized fish was adequate during ocular PO_2 measurements, PO_2 levels in dorsal aortic blood were measured. An indwelling cannula, as for injecting acetazolamide, was used to sample blood. To avoid contact with air, fresh blood was allowed to enter the heparinized cannula; a section (2.5 cm) containing this fresh blood was then clamped and cut free of the cannula. PO_2 was determined by inserting the tip of the oxygen microelectrode into the cannula section which was suspended in a water bath (15°C).

During optimal gill irrigation, the PO_2's of blood from the dorsal aorta were close to those measured in unanesthetized fish swimming freely in the tank. Values ranged from 100 to 135 mm Hg. These levels compare favorably with data for rainbow trout (Holeton and Randall 1967). When the aortic blood PO_2 was low, as occurred in several experiments, it returned to normal when the gill irrigation was improved. Because the ocular PO_2 level probably depends on arterial PO_2, adequate gill irrigation is essential for obtaining valid measurements of ocular PO_2.

Fish were judged to be blind, in comparison with normal animals, if they did not react to movements above the tanks and to shadows falling over the water.

Results

Visual Pigment Density

Among all retinal extracts, the wavelength (λmax) of the maximum change of absorbance (ΔA) occurred between 509 and 515 nm. The variation in the λmax among the individual fish is due to differences in the proportions of the two rod pigments, rhodopsin and porphyropsin. The small range (6 nm) in the λmax values for the retinal extracts is not of sufficient magnitude to interfere with an interpretation of the results even though rhodopsin has a higher molar extinction coefficient than porphyropsin. The following comparisons are therefore made with respect to maximum absorbance changes of the visual pigment in retinal exracts of uniform volume (0.5 ml) when totally bleached. These maximum absorbance changes in retinal extracts from dark-adapted fish that were previously light adapted following pseudobranchectomy or acetazolamide injection are considered to be an indication of the retina's ability to regenerate rod pigments.

Retinal extracts of dark adapted control fish had an average maximum absorbance change (ΔA) following bleaching of 0.170 U/eye, whereas it was 0.035 U/eye for controls that were not dark adapted prior to eye removal (Table 1). Following bilateral pseudobranchectomy (B.P.), there was a progressive decrease in the amount of visual pigment that regenerated during a 1-h period of dark adaptation (compare values at 3, 6, 9, 12, and 24 h with those of dark-adapted controls, Table 1). By 6 h it was significantly less than in dark-adapted controls and by 24 h it was significantly less than light-adapted controls (significant difference between two means is accepted at the 5% level using Student's *t*-test for paired and unpaired observations). By 72 h, there was essentially no extractable visual pigment and the condition persisted.

Fish with unilateral pseudobranchectomy (U.P.) gave different results (Table 1). Removal of the right pseudobranch did not result in a progressive decrease in the amount of visual pigment that regenerated in the right eye during dark adaptation (compare U.P. values with B.P. values at 6, 12, 24, and 168 h following pseudobranchectomy). However, there were significantly lower amounts of visual pigment regenerated in the right and left eyes at 6 and 12 h and in the right eye at 168 h after removal of the right pseudobranch compared with dark-adapted controls. This suggests that unilateral pseudobranchectomy had some initial effect on both eyes and it persisted in the ipsilateral eye. Likewise if the right pseudobranch and the left eye were removed at the same time there was no marked decrease in the density of the extract from the right eye (Table 1). When the left pseudobranch was also removed 24 h after removal of the right pseudobranch, the density of visual pigment in the right eye was comparable to that of other B.P. fish at 24 h following pseudobranchectomy.

In all fish, bilateral pseudobranchectomy caused complete body darkening and blindness within 2–3 h, whereas unilateral pseudobranchectomy had no such effect, even when one pseudobranch and the contralateral eye were removed. This indicates that the ipsilateral eye was intact and functional. Similar body darkening can also be induced by removal of both eyes or by preventing light from reaching the retina. In the case of bilateral pseudobranchectomy, however, it is most likely an impairment of the visual system at the level of the retina rather than a blocking of light reaching the retina that causes blindness and subsequent body darkening.

An intramuscular injection of 25 μg of acetazolamide caused complete body darkening and blindness within 2 h and a significant decrease in

TABLE 1. Maximum absorbance changes (ΔA) in retinal extracts (0.5 ml) of eyes from Atlantic salmon. ΔA represents the mean (range) expressed as 1 retina/0.5 ml of extract.

		No.	Time following pseudobranchectomy or injection	ΔA
Control	Dark adapted	15		.170 (.160–.181)
	Light adapted	5		.035 (.026–.056)
Bilateral pseudobranchectomy	Dark adapted			
		5	3 h	.141 (.124–.161)
		5	6 h	.124 (.110–.140)
		5	9 h	.108 (.090–.118)
		5	12 h	.085 (.079–.090)
		5	24 h	.015 (.012–.022)
		5	28 h	.017 (.014–.023)
		5	72 h	.004 (.001–.009)
		5	168 h	.008 (.002–.011)
Right unilateral pseudobranchectomy	Dark adapted			
		5	6 h	.140 (.121–.165) right eye .152 (.125–.168) left eye
		5	12 h	.150 (.132–.159) right eye .154 (.138–.161) left eye
		5	24 h	.177 (.159–.192) right eye .203 (.186–.211) left eye
		5	168 h	.153 (.141–.167) right eye .194 (.169–.208) left eye
Left eye and right pseudobranch removed	Dark adapted			
		5	120 h	.210 (.195–.219) right eye
		5	14 days	.177 (.169–.192) right eye
Left eye and right pseudobranch removed, left pseudobranch removed 24 h later	Dark adapted	5	24 h	.034 (.030–.041) right eye
Acetazolamide injected (25 μg) intramuscularly	Dark adapted	5	12 h	.090 (.079–.098)
		5	24 h	.174 (.160–.188)

the amount of visual pigment at 12 h postinjection. The effect was not permanent, however, and by 24 h postinjection the visual pigment density was comparable to that of dark adapted controls (Table 1). At 24 h postinjection, the degree of body darkening had decreased, although the fish still appeared to be blind. These fish had very little body darkening 48 h after injection and none appeared to be blind. Thus, the effect of the acetazolamide injection, although initially comparable to that of bilateral pseudobranchectomy, did not cause a permanent loss of the capacity to regenerate visual pigment, permanent blindness, and body darkening.

NORMAL OCULAR PO_2 LEVELS

Oxygen tensions in the eyes of seven unoperated, uninjected salmon were well above air saturation, especially near the retina, where values between 300 and 500 mm Hg were commonly found. Representative results for two of these fish are shown in Fig. 1 and 2. Away from the retina, the O_2 tension dropped markedly to near or slightly below air saturation. The steepest part of the gradient was generally within 1 nm of the retina. This was especially clear in those experiments where PO_2 was measured at frequent intervals within the vitreous humor (Fig. 3).

Oxygen tension in the eye was largely dependent on the supply of oxygen to the animal. This was observed following operative procedures in which the fish was out of the water for several minutes and is illustrated by the data from the sham operation, (Fig. 1). Ocular PO_2's measured shortly after the operation, although quite high, are lower than control values. Ocular PO_2's were also influenced by the effectiveness with which the gills of the deeply anesthetized fish were irrigated during measurements over long periods. In those cases where gill irrigation was inadequate, as judged by low oxygen tension in blood samples from the dorsal aorta, the ocular PO_2 was also low. We effected increases in both dorsal aortic and ocular oxygen tensions under these circumstances by improving gill irrigation or by returning the fish to the recovery tank for a short period.

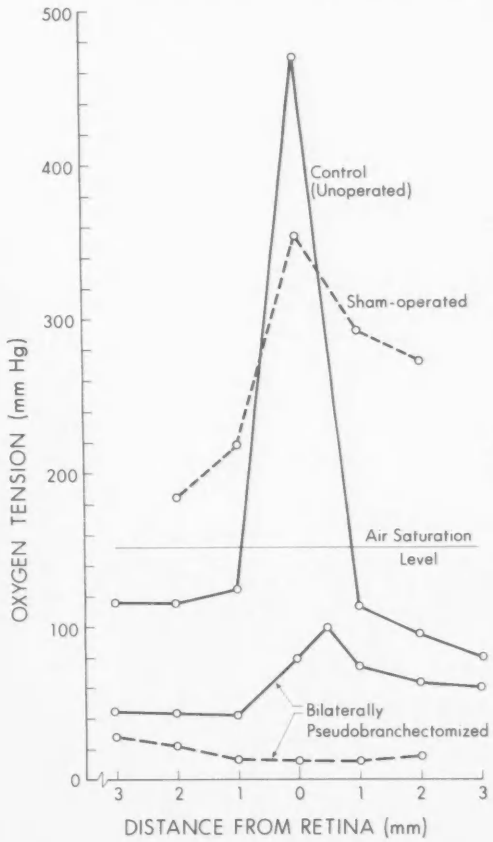

FIG. 1. Representative examples of the effects of bilateral pseudobranchectomy on ocular oxygen tensions in Atlantic salmon. The same fish was used for control, 0.5 h after sham operation, and 1 h after bilateral pseudobranchectomy (upper three curves). The lower curve is for another fish measured 3 h after bilateral pseudobranchectomy.

Sham-operated fish had ocular PO_2's not markedly different from unoperated controls (Fig. 1). These fish retained their normal body color. In the example given, we believe the postoperative depression in PO_2 resulted from inadequate gill irrigation during the operation just completed rather than from effects of the operation itself. Indeed, sham-operated fish, after sufficient recovery time, had PO_2's in the same range as controls.

Effects of Bilateral Pseudobranchectomy (B.P.)

Ocular PO_2 values following bilateral pseudobranchectomy were always markedly lower than those of control and sham-operated fish and never exceeded 65% (99 mm Hg) of air saturation (152 mm Hg) (Fig. 1). These values tended to decrease with time following the operation. Of 14 B.P. fish, 8 had values as low as 10% of air saturation. The effects of the operation, including body darkening, were already very marked after 10 min. PO_2's did not recover during as much as 14 days, the maximum time we observed them. Ocular PO_2 does not decrease to zero probably because some blood reaches the central part of the choriocapillaris via capillaries from the retinal artery, a branch of the internal carotid (Barnett 1951).

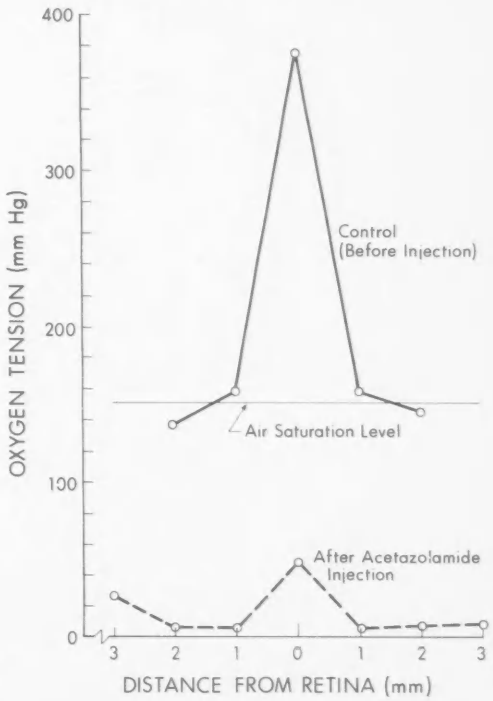

FIG. 2. Representative example of the effects of acetazolamide injection on ocular oxygen tension in an Atlantic salmon. Data are for the same fish before and 4 h after injection.

Effects of Acetazolamide Injections

The effects of acetazolamide injected into the dorsal aorta were similar to those of bilateral pseudobranchectomy. Body darkening and blindness were observed within 15 min after injection. Ocular PO_2 values measured from 4 to 14 h post-injection did not exceed 50 mm Hg (Fig. 2).

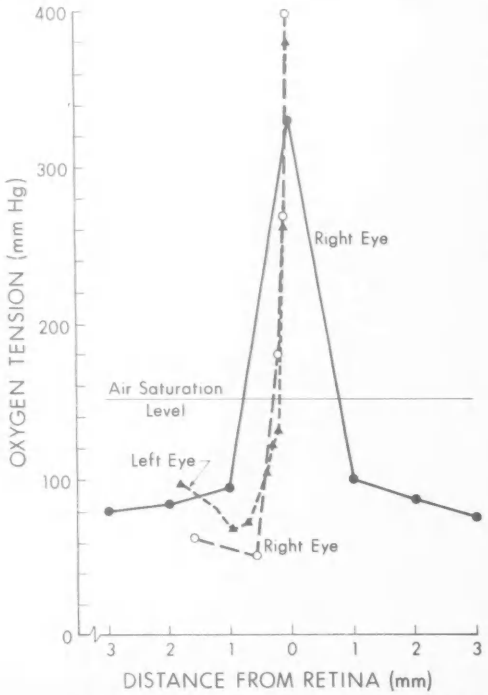

FIG. 3. Representative examples of the effects of unilateral pseudobranchectomy on ocular oxygen tension in Atlantic salmon. Broken curves are for the right and left eyes of the same fish measured at 23 and 22.5 h, respectively, after pseudobranchectomy on the left side. Note similar, normal PO_2 values for both eyes. Solid line is for the right eye of another fish measured 6 h after pseudobranchectomy on the right side. Dorsal aortic PO_2 for the second fish was 95 mm Hg shortly after the ocular PO_2 measurements were made.

EFFECTS OF UNILATERAL PSEUDOBRANCHECTOMY (U.P.)

Ocular PO_2's in U.P. fish were always higher than those of B.P. and acetazolamide-treated fish. There was no marked difference between values for ipsilateral and contralateral eyes (Fig. 3). Oxygen values as high as those in unoperated fish were recorded. The variation in maximum oxygen tensions in seven of the U.P. fish was greater than for control fish, probably owing to postoperative hypoxia, which was not a factor in controls.

Discussion

When light-adapted, bilaterally pseudobranchectomized Atlantic salmon are dark adapted for 1 h, there is a substantial regeneration of visual pigment even though ocular oxygen tensions are very low. This is shown by the fact that following light exposures of 3, 6, 9, and 12 h after bilateral pseudobranchectomy (B.P.), which would bleach most of the rod visual pigment, subsequent dark adaptation results in a significant increase in the amount of visual pigment (Table 1). In comparison, oxygen tensions in the eyes of bilaterally pseudobranchectomized fish decreased precipitously (to as low as 10% of air saturation) within an hour of the operation. There is, nontheless, a progressive decrease in the density of retinal extracts in B.P. fish and by 24 h the amount of extractable visual pigment is less than that of light-adapted controls. This suggests that visual pigment regeneration in the retina is ultimately dependent on a continual blood supply from the choroid rete and quite possibly on the high ocular oxygen tensions associated with this supply. We have no basis for suggesting whether it is a prolonged lack of oxygen or the general loss of other essential blood-borne factors that is responsible for the decrease in the capacity to regenerate visual pigment. Also, B.P. fish were very dark and blind, as judged by their behavior (see Denton and Saunders 1972), within 2 h even though a substantial amount of visual pigment regenerated. This suggests that the initial blindness and the resulting body darkening is caused by an impairment of retinal function other than a loss of ability to regenerate visual pigment during the visual cycle.

Extracts of the right eye of fish, with only the right pseudobranch removed, had visual pigment densities comparable to those of the left eye and of control eyes. This finding disagrees with that of Fairbanks et al. (1969) who concluded that "unilateral pseudobranchectomy results in functional removal of the choroidal rete mirabile of the ipsilateral eye." In Atlantic salmon dissection confirmed the presence of a connection between left and right efferent pseudobranchial vessels (ophthalmic arteries) just anterior to the circle of Willis (see Goodrich 1958). Thus, the choroid rete of the ipsilateral eye can receive blood from the contralateral pseudobranch following unilateral pseudobranchectomy. As will be discussed later, this interconnection is responsible for normal oxygen tensions being maintained in both eyes of unilaterally pseudobranchectomized salmon.

Although acetazolamide caused a significant decrease in the amount of visual pigment within 12 h of intramuscular injection, by 24 h the amount of visual pigment was comparable to that of controls. Likewise, the body darkening and blindness did not persist beyond 48 h. Therefore, this inhibitor of carbonic anhydrase is inactivated or excreted allowing the carbonic anhydrase dependent systems to return to normal functioning (see following discussion on oxygen concentrating mechanism).

Fairbanks et al. (1969, 1974) and Wittenberg and Wittenberg (1974) found high ocular PO_2 values near the retina of several teleosts. Furthermore, Fairbanks et al. (1969, 1974) reported that in rainbow trout, *Salmo gairdneri*, the ocular PO_2 is reduced drastically following intramuscular acetazolamide injections. It reached a level close to the oxygen pressure they measured in dorsal aortic blood (25 and 21 mm Hg, respectively). After unilateral pseudobranchectomy these authors found a partly reduced oxygen pressure in the contralateral eye (239 against 445 mm Hg in unoperated fish) and a markedly reduced retinal PO_2 in the ipsilateral eye (57 mm Hg).

From these results they conclude that (1) the choroidal rete mirabile is an important oxygen-concentrating mechanism; (2) when the choroidal rete mirabile is inactivated through unilateral pseudobranchectomy, oxygen is concentrated in a secondary structure, the lentiform body; (3) carbonic anhydrase plays an important role in the oxygen-concentrating mechanism.

Ocular PO_2 values that we observed in unoperated Atlantic salmon are in general agreement with published data of Fairbanks et al. (1969, 1974) and Wittenberg and Wittenberg (1974). Injections of acetazolamide gave similar or lower ocular PO_2 values for Atlantic salmon than Fairbanks et al. observed in rainbow trout. Fairbanks et al. concluded that carbonic anhydrase is essential for the oxygen-concentrating mechanism on the grounds of their observation that the retinal PO_2 in acetazolamide-treated fish is close to dorsal aortic PO_2. That their retinal PO_2 values are slightly higher than the arterial blood PO_2, together with the presence of an oxygen diffusion gradient between blood and retina, suggests that oxygen concentration still takes place in spite of carbonic anhydrase inactivation. The degree of carbonic anhydrase inhibition in our study and those of Fairbanks et al. is unknown. However, there is reason to believe that the dorsal aortic blood PO_2 values of Fairbanks et al. are not representative of normal arterial blood. Holeton and Randall (1967) observed values as high as 122 mm Hg as against 22 mm Hg (Fairbanks et al.) for the same species. In our work and that of Holeton and Randall, the dorsal aortic PO_2 measurement was made directly using an indwelling cannula while the fish was unrestrained in a tank or while its gills were irrigated artificially. Fairbanks et al. (1969) state that the blood was taken from the dorsal aorta using a hypodermic syringe but do not mention the breathing conditions of the fish. Holeton and Randall found very rapid and marked changes in dorsal aortic PO_2 when the O_2 supply to the gills is reduced. Moreover, the mineral oil used by Fairbanks et al. might have reduced the sensitivity of their O_2 electrode. On the basis of our results showing that the ocular PO_2 following acetazolamide injection is markedly lower than in arterial blood, we present further strong evidence for the conclusion that carbonic anhydrase is essential for the oxygen-concentrating mechanism in the fish eye. This is in agreement with the observation (Fairbanks et al. 1974) that carbonic anhydrase inhibitors can be detected, after intraperitoneal administration to the fish, in organs that are believed to play a role in oxygen concentration. Unfortunately, we did not measure ocular PO_2 beyond 14 h postinjection to determine whether the PO_2 would eventually return to normal levels.

There is a discrepancy between our data and those of Fairbanks et al. (1969) for the effects of unilateral pseudobranchectomy (U.P.). Their values for the contralateral eye, although higher than air saturation, were still markedly lower than ocular PO_2's in unoperated fish. Their readings for the ipsilateral eye were reduced to 57 mm Hg. However, our data for U.P. fish show no such difference between PO_2 in ipsilateral and contralateral eyes. The values measured in both eyes of the salmon were nearly identical with those in unoperated, control fish. This is in agreement with the observation that U.P. salmon did not darken in color and did not show behavioral blindness.

Fairbanks et al. (1969) concluded that the relatively high PO_2 in the ipsilateral eye of U.P. fish was maintained by the lentiform body which receives blood, not from the pseudobranch, but from a branch of the internal carotid (Barnett 1951).

To determine whether the lentiform body has such a marked influence on the PO_2 in the eye we extended the experimental procedure to include bilateral pseudobranchectomy. If the lentiform body played such a major role, then B.P. fish should also have a relatively high ocular PO_2, but they did not. B.P. results in a permanent and dramatic reduction in ocular PO_2. This effect is comparable with that following acetazolamide injection. Likewise, if the lenitform body plays a major role in maintaining relatively high ocular PO_2 even in the absence of the pseudobranchs (and thus the choroid rete), we would expect that acetazolamide-injected fish with all the carbonic anhydrase in the body, including the lentiform body, inhibited, would have markedly lower ocular PO_2 values than B.P. fish, but they did not. We believe that the high PO_2 in the ipsilateral eye of the U.P. fish is due to blood from the intact pseudobranch crossing over to supply the choroid rete of this eye via the interconnection between the ophthalmic arteries.

Wittenberg and Haedrich (1974) suggest that the pseudobranch's function in relation to the choroid rete is to maintain low bicarbonate levels in the blood supply to the choroid rete. This would be important in preventing a rise in carbon dioxide tension in the eye during countercurrent multiplication in the choroid rete. In support of this view they cite the experiment of Maetz (1956), who measured pH in the vitreous humor near the retina of the perch (*Perca fluviatilis*). There were decreases in pH from a normal value of 7.6–6.9 in an hour and to 6.6 by 12 h after acetazolamide injection. Presumably what happens on loss of carbonic anhydrase activity, through the action of the specific inhibitor acetazolamide, is that tissue and blood PCO_2 increase. This increase together with the concentrating effect of the choroid rete presumably results in a lowered pH. This drop in pH impairs retinal function and the fish become blind. Thus, Wittenberg and Haedrich (1974) offer an attractive hypothesis — that "the pseudobranch acts in consort with the choroid rete mirabile to create a high oxygen concentration at the retina without simultaneously accumulating an excessive concentration of carbon dioxide."

Fairbanks et al. (1974) offer an hypothesis on the mode of action of retinal and choroidal carbonic anhydrase in the oxygen-concentrating mechanism in the teleost eye. They suggest that retinal carbonic anhydrase via the dissociation of carbonic acid results in an increase in plasma PO_2 via Bohr and Root effects. The choroidal rete carbonic anhydrase is distributed on the luminal surface of the choroid rete endothelium or in the endothelial cells. Carbon anhydrase could conceivably come from the pseudobranch as has been suggested by Copeland (1951) on the basis of experimental results; however, this was later contradicted by Maetz (1956). In the venous rete capillaries, it catalyzes the hydration of CO_2, preventing the diffusion of this gas to the arterial side where it could cause diffusion of O_2 from the afferent to the efferent vessels, in which case the retina would rapidly be depleted of oxygen. This is in agreement with the observations that ocular PO_2 decreases very rapidly after injection of carbonic anhydrase inhibitors.

Although these hypotheses are reasonable, there are several questions to be answered. Among these are (1) Does the pseudobranch secrete carbonic anhydrase or in some other way (pH, ionic composition, gas tensions) modify the blood before it goes to the choroid rete? (2) Does the choroid rete itself produce carbonic anhydrase? (3) Do those freshwater fish with well-developed choroid retia but lacking pseudobranches (see Wittenberg and Haedrich 1974) have high ocular PO_2 levels? (4) What is the primary cause of blindness following bilateral pseudobranchectomy? Initially it is not a lack of visual pigment. Does blindness result from impairment of the transduction process during pigment bleaching? Does B.P. affect the electrophysiological activity of other cells in the retina? (5) Are the factors causing blindness brought about by O_2 deficiency or by pH changes? In any case, the influence is reversible initially because recovery occurs in acetazolamide-injected fish.

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Blood Changes in Atlantic Cod (*Gadus morhua*) Infected with *Trypanosoma murmanensis*

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KHAN, R. A. 1977. Blood changes in Atlantic cod (*Gadus morhua*) infected with *Trypanosoma murmanensis*. J. Fish. Res. Board Can. 34: 2193–2196.

Blood samples for hematological and parasitological studies were obtained from 30 Atlantic cod (*Gadus morhua*) at intervals after infection with *Trypanosoma murmanensis* via leeches. Hematological procedures included estimation of hematocrit, hemoglobin, erythrocyte sedimentation rate (ESR), and differential white cell count. Tissues were also examined for histological changes. Results from the cod could be divided into two groups. In the first group of 10 cod (16–24 cm), coincident with the rise in parasitemia, hematocrit and hemoglobin levels decreased and the ESR increased following infection. Values did not return to normal levels by 92 days postinfection despite decreasing and somewhat irregular fluctuating parasitemias after 29 days. There was no apparent change in the differential white cell count. Increased hemopoiesis occurred in the kidney and various developmental stages of erythrocytic precursors were noted in peripheral blood. The fish appeared somewhat lethargic. In the second group of 20 cod (26–49 cm), hematological changes observed were slight and there was no evidence of decreased mobility. These results suggest that the blood changes observed in the group of small cod are sufficient to affect them similarly under natural conditions so that they could become more prone to predators, pollutants, or diseases.

Key words: blood changes, Atlantic cod, trypanosomes

KHAN, R. A. 1977. Blood changes in Atlantic cod (*Gadus morhua*) infected with *Trypanosoma murmanensis*. J. Fish. Res. Board Can. 34: 2193–2196.

Nous avons prélevé des échantillons de sang pour études hématologiques et parasitologiques sur 30 morues franches (*Gadus morhua*) à divers intervalles après infection par *Trypanosoma murmanensis* par voie de sangsues. Comme protocole hématologique, nous avons utilisé une estimation de l'hématocrite, de l'hémoglobine, de la vitesse de sédimentation des érythrocytes (ESR) et la différence dans le nombre de globules blancs. Nous avons également examiné des tissus en vue d'y détecter des changements histologiques. D'après les résultats, on peut diviser les morues en deux groupes. Dans un premier groupe de 10 morues (16–24 cm), une élévation de parasitémie est accompagnée d'une diminution des niveaux d'hématocrite et d'hémoglobine, alors que l'ESR augmente à la suite de l'infection. En dépit de la diminution et de fluctuations quelque peu irrégulières des parasitémies après 29 jours, les valeurs n'étaient pas encore retournées à leurs niveaux normaux 92 jours après l'infection. Il n'y a pas de changement apparent dans le nombre différentiel de globules blancs. Il y a hématopoïèse accrue dans le rein, et nous observons des précurseurs érythrocytiques à divers stades de développement dans le sang périphérique. Les poissons semblent quelque peu léthargique. Dans le second groupe de 20 morues (26–49 cm), les changements hématologiques sont minimes et il n'y a pas de diminution évidente de mobilité. D'après ces résultats, les changements observés dans le sang du groupe de petites morues suffiraient à les affecter de la même façon dans des conditions naturelles, les rendant ainsi plus vulnérables aux prédateurs, aux polluants ou aux maladies.

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TRYPANOSOMES are known to be pathogens in some mammals; they induce blood changes following natural and experimental infection (reviewed by Losos and Ikede 1972). Hematological changes, however, have not been reported during life cycle studies of trypanosomes in birds (Baker 1956; Bennett 1961, 1970; Molyneux 1973), reptiles (Hoare 1931; Woo 1969a), or most amphibia (Lehmann 1952; Perez-Reyes 1968; Woo 1969b) and infections are presumably nonpathogenic. An exception is *Trypanosoma diemyctili*, which is pathogenic to adult newts (*Triturus v. viridescens*) held at 15°C (Barrow 1958). In fish, Smirnova (1970) reported that *Trypanosoma lotae* decreased hemoglobin, hematocrit, and blood protein levels in ling (*Lota lota*) and increased phagocytic leucocytes. Lom and Suchankova (1974) also reported drastic changes of blood parameters in carp (*Cyprinus carpio*) following infection with *T. danilewskyi*. In light of the latter reports, the present investigation was undertaken to ascertain whether blood changes occur in Atlantic cod (*Gadus morhua*) following transmissions of *T. murmanensis* via infected leeches. The life cycle of this parasite was reported recently (Khan 1976).

Materials and methods — Atlantic cod (16–49 cm) were caught by hook and line off the coast of Newfoundland, near St. John's. They were subsequently placed in tanks (100 × 80 × 65 cm) through which seawater, at −1–5°C, flowed at about 5 ℓ/min. The fish were held for 3 mo prior to infection. During this period their blood was examined for the presence of trypanosomes. Cod that harbored these hemoflagellates or a parasitic copepod (*Lerneocera branchialis*) reported by Mann (1970) to induce hematological changes, or fish showing any form of physical damage or abnormalities were discarded. The fish were fed freshly thawed capelin (*Mallotus villosus*) thrice weekly.

Leeches (*Johanssonia* sp.) were used to transmit the trypanosome infection to cod. Uninfected adult leeches were first placed on a cod harboring an infection. After feeding, they were held at 0–2°C in 500-ml glass jars filled with seawater (changed thrice weekly) until the blood meal was digested. Some of the leeches were then dissected in saline, as reported previously (Khan 1976), to ascertain the presence of metatrypanosomes in their probosces. Five of them were subsequently attached naturally to each uninfected cod to transmit the infection. Similarly, five uninfected leeches per fish were attached to an equal number of cod that served as controls. All fish were held at the ambient water temperature (−1–2°C). Blood was taken at intervals thereafter to determine parasitological and hematological changes. It was obtained from the gills of fish by wiping the gill dry, piercing the latter with a stylet, and collecting it in a heparinized microcapillary centrifuge tube. The punctured area was lightly pressed to constrict blood flow and aid clotting. Mucus was then smeared over the area and the fish replaced in the tank. Any fish that bled on return to the tank was discarded.

Parasitemias were estimated using a hemocytometer. Hematological procedures, as outlined by Blaxhall and Daisley (1973), included measurements of hematocrit, hemoglobin, and erythrocyte sedimentation rate (ESR). Hemoglobin concentration was determined colormetrically with a hemoglobinometer (American Optical Corp.). Since red blood cell concentrations tend to be low in piscine blood, counts from two blood chambers were divided by two to give the final concentration of hemoglobin. In the micro Wintrobe method for determining ESR, a heparinized microcapillary centrifuge tube was filled to about 50 mm with blood, one end sealed, and the tube left in a vertical position for 1 h. The rate of fall was then measured using millimetre graph paper. The same tube was also used for determination of hematocrit. Differential white cell counts were made on blood smears fixed with methanol and stained with Giemsa. To determine changes in the tissues, all fish were eventually killed, and their tissues (heart, liver, kidney, spleen, and brain) were fixed in Bouin's solution, sectioned at 6 μm, and stained with hematoxylin and eosin.

Cod infected with *T. murmanensis* could be divided into two groups: 10 small cod (16–24 cm, 1–2 y old) in which blood changes were observed and 20 larger fish (26–49 cm, 2 y and older) in which changes were not apparent.

Cod (16–24 cm) — Coincident with the appearance of trypanosomes and the apparent rise of the parasitemia, hematocrit and hemoglobin levels decreased in the experimental cod from mean normal values of 24% and 4.75 g/100 ml 10 days postinfection, reaching mean lows of 15% and 2.8 g/100 ml, respectively, from 21 days onward and showed no evidence of recovery at 92 days (Fig. 1). Although parasitemias declined from 29 days onwards, they fluctuated irregularly throughout the course of the infection in all fish. The ESR increased progressively and did not return to normal by 92 days. Control fish showed no striking departure from normal levels and although some overlap of the confidence intervals does occur during the early stages of the infection, the differences become more apparent as the infection progresses. There was no apparent change in the differential white cell count among infected and control cod. Examination of peripheral blood smears, however, revealed an increase of erythrocytic precursors in the infected fish from 21 days onwards. Increased hemopoiesis was apparent in stained sections of head kidney of all fish killed at 92 days postinfection, but none of the organs showed abnormalities. The fish appeared somewhat lethargic from about 26

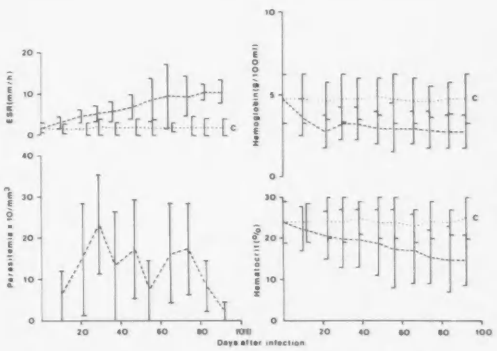

FIG. 1. Mean changes in parasitemia, erythrocyte sedimentation rate (ESR), hemoglobin, and hematocrit (broken lines, with 95% confidence limits) in 10 Atlantic cod (16–24 cm) up to 92 days after infection with *Trypanosoma murmanensis*; c = controls.

days onwards and did not move around the tank as did the controls. Presumably, this decrease in activity is related to the anemia, but the cause of the latter is unknown.

Cod (26–49 cm) — Initially, parasitemias were comparable to those noted in the first group of fish with a peak at 29–30 days but were of short duration and parasites were only seen occasionally after 45 days. Hematological changes, however, were slight. No apparent decrease occurred in the hematocrit or hemoglobin levels and ESR changes were not significant. No change in the differential white cell count occurred and tissues, on microscopic examination, revealed no abnormalities. There was also no evidence of decreased mobility in these fish.

Atlantic cod in their 1st yr of life tend to remain in coastal areas, especially in the bays of Newfoundland (Templeman and Fleming 1965); in this zone, they are generally free of trypanosomes (Khan 1972). The fish commence migration after 1 yr, and as they move offshore they swim into deeper water where they encounter the leech *Johanssonia* sp. that transmits the trypanosome (Khan 1976). Parasites, when apparent in inshore cod older than 1 yr, are generally large and few in number; only one small cod (21 cm) to date harbored a heavy infection made up primarily of intermediate-sized trypanosomes (Khan unpublished data). Data from field studies, however, indicate that parasitemias in young cod (16–24 cm) captured offshore are comparable to those noted in the small cod in the present study and the parasites, small in size, suggest recent infections (Khan unpublished data). Although hematological parameters were not recorded, it is possible these offshore fish undergo changes, similar to those noted in the experimental cod, that affect their activity. If the latter are removed by predators, it is unlikely that they will be encountered in nature. The apparently negative 2-yr-old fish examined inshore might therefore represent those which either have not moved offshore and consequently were not exposed to infected leeches or constitute cod that have recovered. Fish that become infected in their 3rd yr of life or later have a better chance of survival, since infections noted in the older fish (experimental) caused no apparent effects. Possibly, the immune response in these fish is more effective in overcoming the infection than in the young cod.

In areas such as coastal Labrador, where unusually high incidences of trypanosome infections have been recorded in cod (Khan unpublished data), the parasites could have a significant effect on fish numbers. Moreover, the overall impact of trypanosome infections could have deleterious results on the cod stocks, especially if additional stress factors, such as diseases and/or pollutants, are involved.

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Mass Stranding of the Long-Finned Pilot Whale, *Globicephala melaena*, on Sable Island, Nova Scotia

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On December 23, 1976 a herd of over 130 long-finned pilot whales, *Globicephala melaena*, became stranded on Sable Island, Nova Scotia (43° 58'N; 59° 48'W). Data on sex, length, and maximum girth, as well as skin and blubber samples for eventual hydrocarbon analysis were collected on January 15 and 16 from up to 116 carcasses. The herd was composed of at least 65 males and 34 females; 17 animals could not be sexed due to seagull damage. The possible reasons for the stranding are discussed. In particular, implications in the news media that the stranding was precipitated by the December 15 breakup of the oil tanker *Argo Merchant* off the Massachusetts coast are discounted.

Key words: long-finned pilot whale; mass stranding; herd composition; Canadian Atlantic

GERACI, J. R., AND D. J. ST. AUBIN. 1977. Mass stranding of the long-finned pilot whale, *Globicephala melaena*, on Sable Island, Nova Scotia. J. Fish. Res. Board Can. 34: 2196–2199.

Le 23 décembre 1976, un troupeau de plus de 130 globicéphales, *Globicephala melaena*, s'échouaient sur l'île de Sable, Nouvelle-Ecosse (43°58′ de latitude N., 59°48′ de longitude O.). Les 15 et 16 janvier, nous avons cueilli des données sur le sexe, la longueur et la circonférence maximale, de même que des échantillons de peau et de graisse pour dosage éventuel des hydrocarbures, sur un nombre de carcasses allant jusqu'à 116. Le troupeau était composé d'au moins 65 mâles et 34 femelles; les dommages causés par les goélands nous empêchèrent de déterminer le sexe de 17 individus. Nous examinons les raisons possibles de l'échouage. Nous faisons en particulier la part de l'exagération des média d'information dans leurs insinuations à l'effet que la rupture, le 15 décembre, du pétrolier *Argo Merchant* au large de la côte du Massachusetts fut la cause de l'échouage.

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REPRESENTATIVES of every major group of marine mammals strand or are cast ashore. Pinnipeds and mysticete whales strand as single individuals, usually as a result of disease, starvation, or early abandonment as infants. Only the odontocetes sometimes strand in numbers of a hundred or more. (Stephenson 1975; Geraci et al. 1976). Such "mass strandings" have been attributed to natural factors such as inshore

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feeding in dangerously shallow areas (Gresson 1968; Hall et al. 1971), acoustical confusion (Dudok van Heel 1966), hydrological and meteorological conditions (Hall et al. 1971; Stephenson 1975), and unusual disturbances (for example van Bree and Kristensen (1974) attributed a small stranding of *Ziphius cavirostris* in the Caribbean to an underwater explosion). Since whale strandings are the subject of increasing controversy and concern, much of it due to inappropriate reporting and sensationalized media coverage, it is important to document such events critically and objectively. This report deals with a mass stranding of pilot whales on Sable Island, N.S. (43° 58′N; 59° 48′W).

The stranding — On December 23, 1976 at 0920 personnel from the Canadian Atmospheric Environment Service observed a small group of live pilot whales stranded on the north beach of Sable Island. High tide that morning was 0936 (AST) and was 0.9 m above mean water level; peak large high tide is 0.98 m above mean water level. The observers noted that the beach was flooded "beyond levels seen in recent years." Weather conditions for the week preceding the stranding were not unseasonable, although southerly winds shifting between W and SE, and reaching gale force (45 knots) were recorded on December 17 and 21. The seas throughout the period were slight to moderate (Mr B. Hulan, Atmospheric Environment Service, Sable Island personal communication). An aerial survey of the beach, made by representatives of the news media on the afternoon of the stranding (related by P. F. Brodie, Fisheries and Marine Service, Halifax) indicated that at least 130 whales were ashore on a long stretch of the north beach. They were in small groups and concentrated particularly in the area of the original sighting.

Observations and discussion — Our onsite observations on January 15 and 16, 1977 showed 115 pilot whales dead along 25 km of the longer than 30-km north beach, and one whale on the south beach approximately 4 km from the east end of the island (Fig. 1).

There was a sparse, relatively even distribution of 30 whales on the eastern ⅔ of the north beach. Most of the remaining animals were located on a 6-km stretch of beach near the weather station. This was probably the segment of the stranding first reported.

The carcasses were randomly oriented, but in general were parallel to the shoreline, probably the result of wave washing. Some were just below mean high water level; others were well up onto the beaches. There may have been some

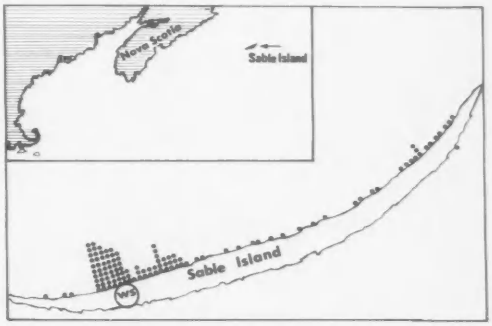

FIG. 1. Distribution of pilot whales stranded on Sable Island. Each dot represents one whale. The major concentration was in the vicinity of the weather station (WS).

redistribution of carcasses at the time these observations were made, but the skin was virtually unmarked in most of the whales, suggesting that such movement of carcasses along the littered sandy beach was minimal. Most carcasses were frozen and many were half buried in sand. Scavenging seagulls were observed feeding at the body orifices, and also on the tissues covering the angle of the jaw (Fig. 2). Their peculiar predilection for this site has been observed in other species (Geraci et al. 1976) and has been er-

FIG. 2. The angle of the jaw is the most common site of seagull damage in both living and dead stranded cetaceans. Such damage has erroneously been attributed to bullet wounds.

roneously attributed to bullet wounds by some observers (Baker and Stephenson 1972).

Of the 99 individuals that could be sexed, there were 65 males (66%) and 34 females (34%) (Fig. 3). Eighteen males (28%) and 25 females (74%) were of sexually mature sizes (Sergeant 1962); there were 7 male and 4 female preweaned calves lacking erupted teeth, and 40 male and 5 female subadults. Seventeen subadult and adult animals could not be sexed due to seagull damage. This group of whales was predominantly male, yet the ratio of adult-sized males to females was 1:1.4. Sergeant (1962) states that a typical mature male to female ratio is 1:3, but acknowledges that there is wide variation in herd composition. It was the subadult males in the Sable Island herd that far outnumbered the subadult-sized females in a ratio of 8:1.

The general body condition of the whales was good; there was no evidence of emaciation. Length–girth relationships were plotted against the only other available data, that from a September 1975 pilot whale stranding in Bonavista Bay, Newfoundland, in which 39 of over 100 stranded whales were examined (Geraci and St. Aubin 1975). There were no major differences (Fig. 3).

The reason(s) for the stranding could not be determined. Sable Island has a long, gentle sloping beach with strong anticlockwise currents. Single strandings of cetaceans occur there frequently (Sergeant et al. 1970). The high tide was near maximum and the north beach was flooded; there were no other extreme environmental conditions for 6 days preceding the event. There were no predators or other sources of harassment observed, but routine observations are seldom made and such occurrences could easily go unnoticed. Due to the frozen condition of the carcasses, no examination could be made of stomach contents to assess inshore feeding behavior.

There were implications in the media that the stranding might have been precipitated by a large oil spill resulting from the December 15 grounding and subsequent breakup of the oil tanker *Argo Merchant* on Nantucket shoals off the Massachusetts coast. By the time of the stranding, the spill had spread in a south-easterly direction toward the southern edge of Georges Bank, nearly 400 naut. miles southwest of Sable Island. There was no evidence of oil on the whales, though skin and blubber samples were collected for hydrocarbon analysis. In view of the considerable distance between the *Argo Merchant* oil and the stranding area, and the lack of any noticeable oil contamination on the carcasses, a causal relationship is extremely unlikely.

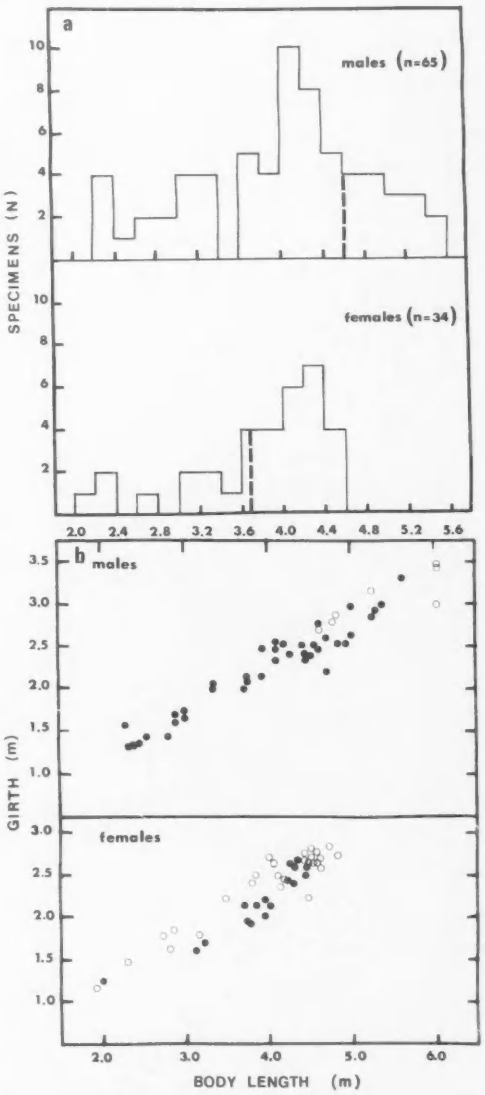

FIG. 3. a) Length-frequency distribution of pilot whales stranded on Sable Island. Broken line represents length at sexual maturity (Sergeant 1962); b) length–girth relationship of pilot whales stranded on Sable Island (●) and Bonavista Bay, Newfoundland (○). Girths could not be determined from all of the Sable Island pilot whales.

Long-finned pilot whale strandings are not unusual occurrences along the eastern North American seaboard (Sergeant et al. 1970; Geraci and St. Aubin 1975). Such events provide a

unique opportunity to gather data on life history, herd composition, diseases, and levels of oceanic pollutants that are otherwise unobtainable.

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Tag Retention of the Spot Prawn, *Pandalus platyceros*, Injected With Coded Wire Tags

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PRENTICE, E. F., AND J. E. RENSEL. 1977. Tag retention of the spot prawn, *Pandalus platyceros*, injected with coded wire tags. J. Fish. Res. Board Can. 34: 2199–2203.

Juvenile spot prawns, *Pandalus platyceros* (initial carapace length 15.0–22.5 mm), were successfully tagged with Bergman–Jefferts coded wire tags in the thoracic sinus. Tagged prawns, prawns subject to tagging needle insertion but without tags, and a control group were tested. No significant differences in growth and survival within or between test groups occurred and no behavioral changes were observed among tagged animals. All tagged prawns molted at least twice during the 6-mo experiment. Average tag retention was 95%.

Key words: *Pandalus platyceros*, tagging, tag retention, molting, survival

PRENTICE, E. F., AND J. E. RENSEL. 1977. Tag retention of the spot prawn, *Pandalus platyceros*, injected with coded wire tags. J. Fish. Res. Board Can. 34: 2199–2203.

Nous avons marqué avec succès de jeunes crevettes, *Pandalus platyceros* (d'une longueur de caparace initiale de 15.0 à 22.5 mm), à l'aide du fil codé de Bergman-Jefferts inséré dans le sinus thoracique. Nous avons fait des essais avec des crevettes marquées, des crevettes soumises à l'insertion de l'aiguille de marquage sans les marques, et un groupe témoin. Il ne se produisit pas de différences significatives dans la croissance et la survie parmi ou entre les groupes d'essais, et nous n'avons observé aucun changement de comportement chez les animaux marqués. Toutes

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les crevettes marquées muèrent au moins deux fois durant l'expérience de 6 mois. La moyenne de rétention des marques fut de 95%.

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VARIOUS methods have been developed for identifying individuals or groups of Crustacea (Neal 1969); however, none have been entirely satisfactory. Tagging and marking can be divided into two classes: external and internal. External tags and markers are not acceptable for they often alter animal behavior, increase mortality, and are shed when the animal molts. Furthermore, small animals cannot be externally tagged. Internal stains are short-term (6 mo maximum) and are often not readily detectable because of animal coloration.

The internal tagging method using the Bergman–Jefferts coded wire tag described by West and Chew (1968) for prawns meets most of the criteria for an acceptable tag. The tag is retained through repeated molts, has numerous code combinations (8×10^4), is relatively easy to apply and detect, and does not alter growth, survival, or behavior of the animal. A major objection to the technique was that the tag was placed in the edible portion (abdomen) of the prawns. We modified the tagging technique by inserting the tag through the anterior portion of the cephalothorax into the thoracic sinus, thus overcoming this objection. This report describes the modified technique and reports the growth, survival, and tag retention of the test prawns.

Materials and methods — Test animals — Juvenile spot prawns, *Pandalus platyceros*, were used in a tagging experiment that lasted from January to July 1976. All test animals were measured from the base of the eyestalk to the posterior middorsal edge of the carapace using calipers at days 0, 104, and 174 of the experiment.

Treatments — Three treatments were evaluated: tagged prawns; wounded prawns — subjected to insertion of a tagging needle but without tags; and a control group — neither tagged nor wounded. Each of the three treatments had five replicates of 30 prawns for a total of 450 animals. Animals were randomly assigned to experimental groups.

Holding containers and animal maintenance — The prawns were held in 15 fiberglass tanks (107 × 27 × 26 cm; volume, 75.1 ℓ). Salt water was continuously supplied to each tank at flow rates varying from 2 to 3 ℓ/min. The water had an average pH of 7.28, a salinity of 28‰, and a temperature range of 7.0–12.0°C. All groups were held under natural photoperiod. The prawns were fed all they would eat once every other day on a diet of salmon, *Oncorhynchus* spp., and mussel, *Mytilus edulis*. Each tank was examined daily and exuviae and dead prawns were immediately removed. To ensure uniformity of exuviae count data, only the carapaces were counted, because this was found to be the last portion of the exuviae to be consumed by the prawns after molting.

Tagging method — Tagging was done with a Technical Research Company wire tag injector, model No. 3 and a Northwest Marine Technology hand-operated tagger. Tag detection was accomplished using a Jefferts electronic closed head tag detector. The mechanics and operation of the tagger and detector were similar to those described by West and Chew (1968). The tags were injected into the thoracic sinus (hemocoel) by penetrating the right-hand frontal region of the cephalothorax approximately at the intersection of lines drawn from the suborbital spine and the 3rd or 4th posterior rostral spine (tooth) (Fig. 1). Care was taken to avoid penetrating vital organs during the injection procedure. Immediately after tagging, the prawns were passed over a magnet to magnetize the tag and then through the tag detector to verify tag insertion; if the tag was not detected (i.e., the detector was not activated) the animals were rejected and not used in the experiment.

Analysis of data — Growth and survival data were analyzed using the *F*-statistic. For each test group and each selected time interval, molt frequency was estimated by ratio E_i/N_i, where i = the selected time interval (1 day or several days) used; the time intervals are monotonic and nonoverlapping; E_i = the number of molts in time interval i; N_i = the number of animals available to molt in time interval i. For example, if a 5-day time interval were used, the above ratio would be calculated as the quotient of the number of molts in the interval divided by the number of prawn alive and available to molt in that interval.

These interval ratios are used to calculate

$$M_t = \sum_{i=1}^{t} (E_i/N_i)$$

where t = the total number of time intervals and M_t = the estimated molt occurrence at the termination of t time intervals.

Results and Discussion — The mean length and standard deviation of prawns for each treatment and growth period are given in Table 1. The initial carapace length ranged from 15.0 to

FIG. 1. Coded wire tag within the thoracic sinus of a spot prawn (tagged for 157 days). A portion of the cephalothorax has been removed to expose the tag.

TABLE 1. Size of prawns in experimental groups at start of study and after 104 and 174 days. CL = carapace length (mm) expressed as mean with SD in parentheses.

	Start of study 0 days		104 days		174 days	
Treatment	Total no.	CL	Total no.	CL	Total no.	CL
Control	150	19.4(1.55)	126	21.3(1.68)	109	22.1(1.52)
Wound	150	19.6(1.45)	128	21.3(1.59)	110	22.4(1.47)
Tag	150	19.7(1.46)	130	21.2(1.39)	85	22.2(1.40)

[a]One replicate ($n = 27$) deleted for histological examination of prawns after 157 elapsed days.

22.5 mm. No significant differences in growth or survival within or between test groups (Table 2) were shown at any time ($F = 0.959$, df = (2,327), $P > 0.25$). Likewise, no behavioral differences were seen between test groups.

Tags were removed from the prawns after either 157 or 180 days and examined microscopically. No alteration to the tags was noted. The tags were found either free floating within the thoracic sinus or within the musculature in the immediate area of tag insertion (Fig. 1). No tags were found in organs. Histological examination of tissue and organs in the area near the tag showed no inflammatory response. The only perceptible damage from tagging was at the point of needle entry. A black spot (melanin-like

TABLE 2. Total elapsed days, survival, and tag retention (values expressed as means with SD in parentheses) for three experimental populations of spot prawns after 1, 2, and 2+ molts.

Treatment	Start of study: No. prawns	Start of study: No. reps.	1 molt: Total elapsed days	1 molt: % Survival	1 molt: % Tag retention	2 molts: Total elapsed days	2 molts: % Survival	2 molts: % Tag retention	2+ molts: Total elapsed days	2+ molts: % Survival	2+ molts: % Tag retention	2+ molts: Estimated no. of molts after 180 days
Control	150	5	93(12.16)	88(11.51)	—(—)	147 (7.03)	77 (6.47)	—(—)	180(0)	71 (5.43)	—(—)	2.71 (4.51)
Wound	150	5	93 (8.24)	90 (4.72)	—(—)	146 (5.60)	80 (4.21)	—(—)	180(0)	66(12.39)	—(—)	2.76(28.30)
Tag	150	5[a]	95(12.75)	90 (2.09)	96(4.19)	157(10.57)	77(10.34)	95(4.79)	180(0)	69(11.41)	95(9.01)	2.63(25.65)

[a]One replicate ($n = 27$) deleted for histological examination of prawns after 157 elapsed days.

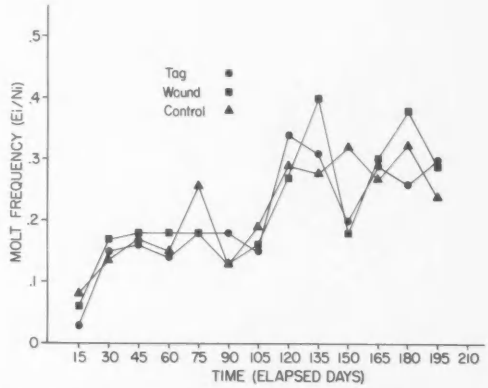

FIG. 2. Molt occurrence for combined replicates of wound, tag, and control groups based on 15-day periods.

deposit) formed at the wound site within a day or two after tagging, but was lost at first molt. The absence of the black spot was used to confirm the first molt after tagging. Penn (1975) described a similar scar formation and used it as a molt indicator.

After 180 days tag retention was 95% and the average molt occurrence was 2.63% for the five tag replicates (Table 2). Tag loss is thought to have occurred primarily through the puncture in the carapace prior to the first molt. Due to surface tension, the tag had a tendency to follow the hypodermic needle as it was retracted from the wound, and tags were seen partially protruding into the puncture. This problem was subsequently overcome in other tests by tapering the tip of the push-rod within the hypodermic needle (for a detailed description of the tagging apparatus see West and Chew (1968)). As a result of this modification, the push-rod inserts the tag at an angle from the needle, and consequently the tag does not follow the needle upon its retraction. This minor change improves tag retention.

No mortality could be attributed to the tagging of the prawns (Table 2). Mortality that did occur (31.3%) for all treatments combined was due to cannibalism at the time of molt or poor water quality during water system failures.

Exuviae were removed daily from all containers and the percent molt occurrence calculated with the aforementioned formula. The substantial decrease in time between molt periods (0 to 1 and 1 to 2) (Table 2) was probably due to the rise in water temperature from 7 to 12°C and/or extended photoperiod. No definite molt cycle could be determined for any treatment group during the study (Fig. 2). There was a trend, however, toward a 60-day cycle on an individual

replicate basis irrespective of treatment. The absolute time of molt peaking would vary between replicates, but the basic 60-day cycle persisted. The variable lighting conditions within the laboratory may account for the variability between replicates.

The Bergman–Jefferts wire tag and the tagging technique described above appear to have considerable merit. First, the tag and tagging technique is applicable to animals of a small size. For example, the minimum carapace length of prawn in our study was 15 mm; however, animals as small as 10 mm have been successfully tagged in subsequent tests. Also, this method of tagging can be applied to several types of crustaceans, such as shrimp, crab, and lobsters. Since the tag is inserted in nonedible portions of the animals, there is no concern over accidental ingestion by consumers.

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Selection of Prey by American Lobsters (*Homarus americanus*) When Offered a Choice Between Sea Urchins and Crabs

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EVANS, P. D., AND K. H. MANN. 1977. Selection of prey by American lobsters (*Homarus americanus*) when offered a choice between sea urchins and crabs. J. Fish. Res. Board Can. 34: 2203–2207.

American lobsters (*Homarus americanus*) were presented with various numerical combinations of sea urchins (*Strongylocentrotus droebachiensis*) and rock crabs (*Cancer irroratus*) in long-term laboratory feeding experiments. They exhibited a 5:1 preference for crabs when the numerical ratio of urchins to crabs varied from 0.25:1 to 8:1. Lobsters presented with a superabundance of sea urchins (urchin:crab ratios of 16:1 and 100:1) maintained relatively constant proportions of urchins and crabs in their diet. Direct observation indicated that lobsters feed selectively. Their role as key predators in kelp–sea urchin communities is discussed.

Key words: food preference, predation, Canadian Atlantic, bioenergetics

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Dans des expériences à long terme d'alimentation en laboratoire, on a présenté à des homards américains (*Homarus americanus*) diverses combinaisons numériques d'oursins communs (*Strongylocentrotus droebachiensis*) et de crabes-tourteaux communs (*Cancer irroratus*). Les homards montrent une préférence de 5:1 pour les crabes quand le rapport numérique d'oursins à crabes varie de 0.25:1 à 8:1. Devant une surabondance d'oursins (rapports oursins: crabes de 16:1 et 100:1), les homards maintiennent dans leur régime des proportions relativement constantes d'oursins et de crabes. L'observation directe indique que les homards exercent un

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choix dans leur nourriture. Nous examinons leur rôle comme prédateurs clés dans les communautés de varechs–oursins communs.

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SEA urchins have destroyed kelp beds along many hundreds of miles of Canadian Atlantic coastline, and in those same areas lobster stocks have declined (Mann 1977). It has been suggested that lobsters are key predators on sea urchins and when lobster stocks decline they allow sea urchin populations to get out of control and destroy the kelp beds on which they feed (Mann and Breen 1972; Breen and Mann 1976). Rock crabs also prey on urchins, but are themselves the prey of lobsters. Breen (1974) showed by means of a model that lobsters could control sea urchin stocks, but the model used two assumptions: that crabs and urchins are about equally preferred by lobsters and that prey are taken in direct proportion to their relative abundance. This study is a detailed investigation of those assumptions.

American lobsters (*Homarus americanus*) in tanks were offered choices with different numerical combinations of urchins and crabs. The experiments differed from those reported earlier (Reddin 1973; Breen 1974) in that the lobsters were given a particular choice of prey for a much greater length of time, thus allowing dietary requirements for nutrients and energy to influence the results.

Materials and methods — For prey choice experiments, 24 freshly caught lobsters of carapace length 92–115 mm were purchased locally and had their claws banded, rather than pegged, to prevent damage to the claw muscles. Each lobster was placed in a tank of 1 m^2 with a drain tile for a shelter. Seawater was pumped locally from a depth of 10 m and was kept at a depth of 20 cm in the tanks. Light was on a 12:12 light–dark cycle with the light phase starting at 0800. To prevent habituation to either prey and to maintain a uniform level of hunger, they were fed on a maintenance ration of mackerel for 1 wk. They were then divided into six groups of four and each group given one of the eight different combinations of prey (Table 1). Numbers and weight consumed of each prey were recorded for 6 wk with daily replacement of killed or unhealthy animals. The experiments were continued until there had been eight replicates for each combination. The results from two lobsters that molted and one that died during the experiments were not used. Prey densities varied between 12 and 36 individuals per tank except in the two highest urchin abundances, which required 101 and 102 prey per tank (Table 1). For direct observation of feeding behavior, lobsters were conditioned for 2 wk at 12°C to a 12:12 light–dark cycle with the dark phase beginning at 1000, and fed a maintenance ration of mackerel, beef liver, clam, or squid. In this way they were made active during the day and could be observed in a lightproof room under a red light, which does not disturb them. (Kennedy and Bruno 1961). Lobsters were observed hunting at urchin:crab ratios from 0.25:1 to 8:1. Two different animals were used each day for 5 days, giving 10 replicates per combination. No pair of lobsters was used more than twice in 1 wk; if a lobster stopped feeding during the experiment it was not used until 3 days after it had resumed feeding. Predatory behavior was recorded in terms of (1) encounters, (2) recognition, (3) pursuits, (4) successful pursuits, (5) captures, and (6) successful captures, i.e. kills.

Results — In a preliminary experiment it was shown that pegging the claws of a crab did not render it more susceptible to lobster predation. In subsequent experiments the claws of the crabs were pegged to prevent them attacking the urchins.

The main group of experiments on choice of prey (Table 1) shows a clear preference for crabs. When results are plotted in terms of either numbers or live weight (Fig. 1) the left-hand parts of the curves have slopes approaching 0.2, indicating a preference of about 5:1 for crabs over urchins. When the ratio of urchins to crabs available is high, the ratio of urchins to crabs in the diet remains relatively constant, about 0.6 g urchin to 1.0 g of crab, or 2 urchins to 1 crab.

In the observation experiments, it was determined that the ratio in which the two kinds of prey were encountered was the same as the ratio of their abundances. The slope of the line in Fig. 2 is not significantly different from 1.0. Data were further analyzed in terms of the equation (Holling 1966)

$$\text{Capture success} = \text{recognition success} \times \text{pursuit success} \times \text{attack success}$$

For urchins an encounter consisted of direct physical contact by a portion of the lobster's body having chemoreceptors. It was considered that all encounters led to recognition, so recognition success was 100%. Attack success was very low, averaging 0.7% with no obvious relationship to urchin relative abundance (Table 2). Hence, for urchins, capture success was $100\% \times 100\% \times 0.7\% = 0.7\%$.

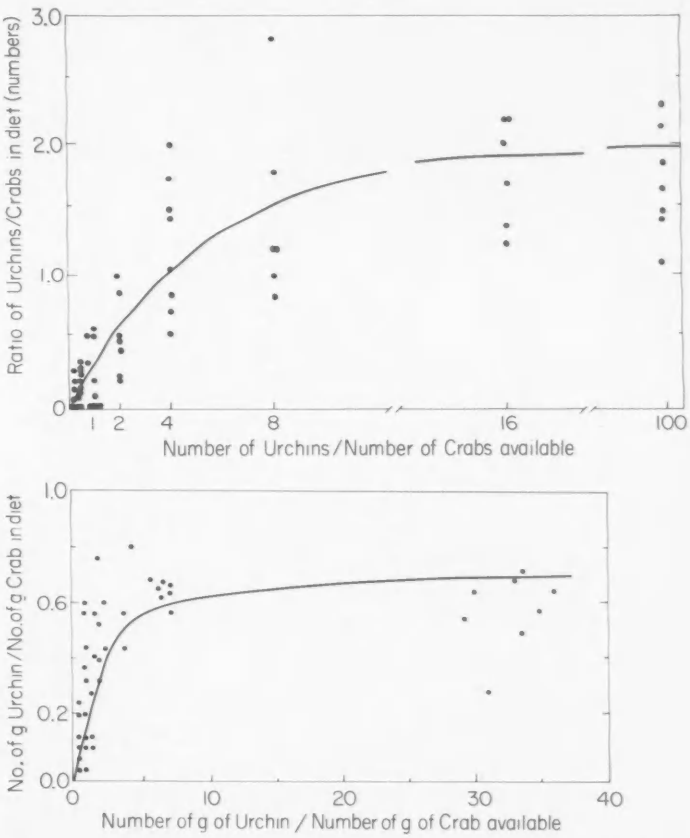

FIG. 1. Relation between ratio of urchins to crabs in the diet and ratio of urchins to crabs in the tanks. Curves fitted by eye. *Upper curve*, numerical ratios; *lower curve*, biomass ratios.

TABLE 1. Lobster predation on mixed population of sea urchins and crabs. Each figure is based on eight replicates except where otherwise indicated.

Total no. of prey	Numerical ratio of urchins:crabs available	Mean ratio urchins:crabs available (g)	Mean no. crabs eaten per wk	Mean no. urchins eaten per wk	Mean numerical ratio of urchins:crabs eaten	Mean ratio urchin:crabs eaten (g)
20	0.25:1	0.10	10.1	0.75	0.07	0.04
12	0.5:1	0.20	7.6	1.90	0.25	0.12
12	1:1	0.38	7.3	1.60	0.21	0.09
12	2:1	0.73	9.3	7.40	0.79	0.42
20	4:1[a]	1.66	9.3	9.50	1.02	0.53
36	8:1[b]	2.75	6.5	13.10	2.01	0.59
102	16:1	6.70	6.8	10.00	1.47	0.66
101	100:1	32.50	6.5	11.50	1.76	0.52

[a]Based on seven replicates.
[b]Based on six replicates.

TABLE 2. Pursuit and capture success of lobsters hunting sea urchins and crabs, and energy obtained from crabs. Experiments conducted from September to December 1975 at an average temperature of 12.5°C. Each figure based on eight replicates except where otherwise stated.

Numerical ratio of urchins:crabs	Capture success of lobsters hunting urchins (%)	Pursuit success of lobsters hunting crabs (%)	Capture success of lobsters hunting crabs (%)	Energy obtained from crabs (cal·g lobster^{-1}·wk^{-1})	Proportion of total energy obtained
0.25:1	0	65	0	75.5	98
0.5 :1	2	62	0	89.7	96
1:1	0	76	6	59.2	98
2:1	1.8	59	14	67.5	90
4:1[a]	0.6	49	4	90.0	89
8:1[b]	0	51	0	54.5	84

[a]Energy data based on seven replicates.
[b]Energy data based on six replicates.

For crabs, recognition success was taken as 100% and pursuit success was 49–76%, with a mean of 60%. Attack success was also low, averaging 4% and with no obvious relationship to relative crab abundance (Table 2). For crabs, capture success was 100% × 60% × 4% = 2.4% and was higher than for urchins by a factor of 3.4. This factor is only approximate, since observations were limited to a few hours of each day, but there is a clear indication that in spite of the mobility of the crabs, the lobsters captured a much higher proportion of crabs than of urchins.

Discussion — The finding that lobsters prefer crabs over urchins raises difficulties in acceptance of the theory (Breen and Mann 1976) that lobsters are the key predators controlling sea urchins in nature. However, there are a number of features of the experiments that make it unwise to extrapolate the results without caution. First, crabs are highly mobile and in nature would more easily escape the lobsters by taking cover under rocks or fleeing into the distance. Neither of these was possible in the tanks. Second, although there are no published data on the population densities of crabs in kelp beds, the densities in the tanks were probably unusually high, whereas the densities of urchins are within the range reported by Breen (1974). Third, the crabs had their claws pegged and were unable to prey on the urchins. In nature they would probably support the lobsters in their role as crab predators.

The experiments indicate that the preference of lobsters for crabs is a real one, even if in nature the lobsters do not always have the opportunity to indulge it. The caloric content of a gram live weight of a crab is 5 times that of an urchin (Breen 1974) and in this study the crabs

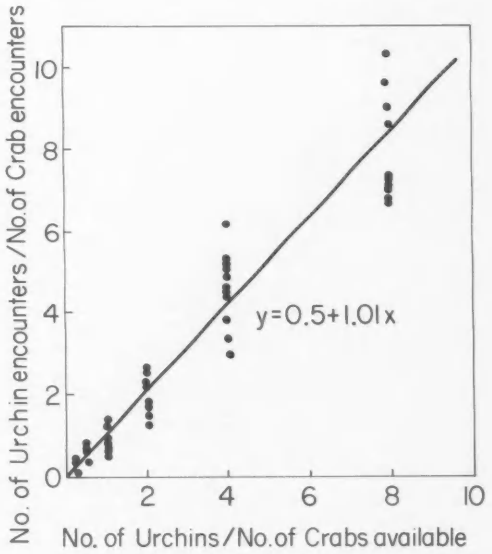

FIG. 2. Relation between ratio of urchin to crab encounters and relative abundances of urchins and crabs. The slope of the fitted regression does not differ significantly from 1.0.

were about 3 times heavier, so that the killing of a crab yielded about 15 times as many calories as the killing of an urchin. On the other hand, more energy is expended in catching a crab. On balance there seems to be a clear bioenergetic advantage in catching and eating crabs. In our experiments the average caloric intake of the lobsters was 66–101 cal/g lobster per wk, of which 84–98% was from crabs (Table 2).

Lobsters have been shown to also have a requirement for a high protein intake (Castell and Budson 1974). In sea urchins no more than

5% of the fresh weight is protein (Giese 1966), while in crabs the proportion is over 20% (Vonk 1960). Moreover, lobsters lacking crustacean material in the diet fail to develop normal coloration (Hughes and Matthiessen 1962). These facts help to explain why lobsters prefer crabs over urchins.

In conclusion, the simple assumptions of Breen's model of lobster predation on crabs and urchins clearly need to be modified. There are good physiological reasons why lobsters should take crabs when they can find them and catch them. This explains the predominance of crab remains in the stomachs of lobsters examined by Weiss (1970) and Ennis (1973). Nevertheless, the study also shows that when sea urchins are very abundant the lobsters take about twice as many urchins as crabs, even under experimental conditions which hinder the escape reactions of the crabs. It is still possible to think of the lobsters as controlling factors in the abundance of urchins, especially if they are supported in this role by the predatory activities of the crabs themselves.

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Evaluation of the Ascorbic Acid Status of Rainbow Trout (*Salmo gairdneri*)

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Hilton, J. W., C. Y. Cho, and S. J. Slinger. 1977. Evaluation of the ascorbic acid status of rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 34: 2207–2210.

Due to the instability of supplemental ascorbic acid in practical trout diets and the increased metabolism of this vitamin in trout ingesting enviromental toxicants, a method of assessing the adequacy of dietary ascorbic acid and the ascorbic acid status of the trout is required. Two groups of trout grown on ascorbic acid deficient diets produced deficiency symptoms within 16–20 wk. Liver ascorbic acid concentrations in the two groups declined to levels below 20 μg/g when deficiency symptoms appeared. Groups of trout grown on diets containing supplemental ascorbic acid to provide near or in excess of the National Research Council–National Academy of Science requirement for this vitamin after processing showed substantially higher liver ascorbic acid

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concentrations than the deficient fish. This indicates that the liver ascorbic acid concentration can be used as an index of the ascorbic acid status of the trout and that levels 20 μg/g or lower are marginal and immediate supplementation is required.

Key words: ascorbic acid, rainbow trout, nutritional requirements

HILTON, J. W., C. Y. CHO, AND S. J. SLINGER. 1977. Evaluation of the ascorbic acid status of rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 34: 2207–2210.

A cause de l'instabilité d'un supplément d'acide ascorbique dans des nourritures à truites et du métabolisme accru de cette vitamine chez des truites absorbant des toxiques du milieu, il est nécessaire d'avoir une méthode permettant d'évaluer la suffisance de l'acide ascorbique dans le régime et l'état de cet acide chez la truite. Deux groupes de truites élevées à des régimes déficients en acide ascorbique produisent des symptômes de déficience en dedans de 16–20 semaines. Les teneurs en acide ascorbique du foie dans les deux groupes baissent à des niveaux inférieurs à 20 μg/g au moment où les symptômes de déficience apparaissent. Les groupes de truites nourries à des régimes contenant un supplément d'acide ascorbique répondant à peu près ou dépassant les normes Conseil national de recherches – National Academy of Science pour cette vitamine après traitement, montrent des teneurs en acide ascorbique dans le foie notablement plus élevées que chez les poissons déficients. Ceci indique que la teneur en acide ascorbique du foie peut être utilisée comme indice de l'état de cet acide chez la truite, et que des niveaux de 20 μg/g ou moins sont marginaux, et qu'un supplément est immédiatement requis.

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RAINBOW trout have a requirement for supplemental ascorbic acid in their diet (Kitamura et al. 1965; Halver et al. 1969). This vitamin is unstable under certain conditions (Hay et al. 1967) and practical trout diets have been shown to lose ascorbic acid during processing and to become deficient or below the NRC (1973) requirement for the vitamin after 5 or 8 wk of room temperature storage, depending upon the type of processing, when initially supplemented with 400 mg/kg coated ascorbic acid (Hilton et al. 1977). This presents the fish culturist or nutritionist with a problem of knowing whether the trout are receiving an adequate level of ascorbic acid in the diet and of determining the ascorbic acid status of the fish. This problem is confounded by the presence of dietary environmental toxicants such as toxaphene which have been shown to increase the ascorbic acid requirement of catfish (Mayer et al. 1977). Measuring the level of ascorbic acid in the diet would thus not necessarily ensure that the concentration found would be adequate for the trout. Therefore, some means of assessing the adequacy of ascorbic acid in the fish is needed.

Two groups of rainbow trout were grown at different times on a practical trout diet similar to diet no. 5 of Cho et al. (1974) except that no supplemental ascorbic acid was added to the diet. These groups developed overt deficiency symptoms 16–20 wk after the start of the experiment. The symptoms noted were anorexia, lethargy, and prostration. Scoliosis and lordosis, although not major symptoms initially, became a dominant part of the syndrome as the deficiency state advanced. Both groups were fed a control diet (400 mg/kg supplemental ascorbic acid) for at least 4 wk prior to the start of the experiment and thus the trout began the experiment with a reasonable store of ascorbic acid. Liver ascorbic acid levels of the two groups, as measured by the method of Aeschbacher and Brown (1972), 8 and 4 wk before overt deficiency symptoms were noted (Table 1) were variable; however, the trend in both groups was a declining liver ascorbic acid concentration from +20 μg/g. The differences in the rates of decline in the two groups may be partially explainable on the basis that while the two groups were fed the same formula on paper, it contained ingredients in the two experiments that may have been contaminated with different levels of environmental toxicants that have been shown to increase the ascorbic acid requirements of fish (Mayer et al. 1977). Most herring fish meals and oils are contaminated to a greater or lesser degree with chlorinated hydrocarbons. Furthermore, the groups were from different lots of fish and were not of similar size when the experiments were begun. The two groups could thus have had totally different storage levels of ascorbic acid at the start of the experiments. Despite these potential differences the two groups showed liver ascorbic acid levels that declined similarly to approximately 12–14 μg/g at the time the overt deficiency symptoms were noted.

Liver ascorbic acid concentrations of trout grown in the same experiment as group 1 of Table 1, but fed diets near or in excess of the NRC (1973) requirement for the vitamin, as measured by the method of Aeschbacher and

Brown (1972), showed substantially higher liver concentrations of ascorbic acid than the non-supplemented groups and did not develop symptoms of deficiency (Table 2). The diets used for the groups in Table 2 were stored at room temperature for at least 5 wk and recent research (Hilton et al. 1977) has shown that storage losses of ascorbic acid under these circumstances were considerable. However, as the experiment progressed, the time period for the processing and storage of diets was reduced. Therefore, the fish sampled in groups 3–5 at the time overt deficiency symptoms were noted in group 1 were receiving a much higher dietary level of ascorbic acid than those fish sampled in these same groups 4 and 8 wk before the symptoms appeared. The higher liver ascorbic acid concentrations noted in groups 3–5, at the time overt deficiency symptoms appeared in group 1, as opposed to the levels found in these same groups 4 and 8 wk before the symptoms appeared (Table 2), thus reflect the increased dietary level of ascorbic acid remaining in these diets.

TABLE 1. Liver[a] ascorbic acid concentrations (micrograms per gram wet weight) of rainbow trout fed non-supplemented diets, 8 and 4 wk before and during overt deficiency symptoms.

Group[b]		Wk before symptoms		Symptoms present
		8	4	
1.		14.7	12.8	9.9
		16.6	13.8	13.7
		18.8	16.2	17.7
	Avg	16.7±2.0	14.2±1.7	13.7±3.8
2.		19.2	18.7	10.5
		29.1	22.1	11.1
		33.5	28.6	13.4
	Avg	27.2±7.3	23.2±5.0	11.6±1.5

[a]Values presented are for pooled samples of four–six livers per sample.

[b]Group 1: body wt at symptoms, 9706±47 g/100 fish; water temp, 15°C ± 0.58, dissolved oxygen, 8.4–7.2 mg/ℓ; pH, 7.7–7.8 mortalities, 2.8/10,000 fish days. Group 2: body wt at symptoms, 7883±243 g/100 fish; water temp, 15°C±0.23; dissolved oxygen, 8.4–7.1 mg/ℓ; pH, 7.7–7.8; mortalities, 1.2/10,000 fish days.

TABLE 2. Pooled liver ascorbic acid concentrations (micrograms per gram wet weight) (four–five livers per sample) in rainbow trout receiving diets supplemented with ascorbic acid.

Group	Diet ascorbic acid level after processing (mg/kg ± SD)	Wk before overt symptoms in groups 1 and 2		Overt symptoms absent
		8	4	
3	83±7	23.0±5.2	27.9±5.7	36.9±2.1
4	168±10	29.7±6.8	33.3±8.4	53.0±5.6
5	201±15	55.3±2.6	46.1±4.8	80.1±6.7

Thus, an assessment of the dietary level of ascorbic acid and the ascorbic acid status of the trout is apparently possible by measuring the liver ascorbic acid concentration. If the liver ascorbic acid concentration is 20 μg/g or less, the ascorbic acid status of the trout is marginal or poor, the dietary level of ascorbic acid is inadequate, and immediate supplementation is recommended.

Despite the fact that both groups of trout fed the unsupplemented diets required a minimum of 16 wk before overt deficiency symptoms appeared, it should not be assumed that this is the period of safety before action is required. Factors such as injuries to the fish (Halver 1972a) and environmental toxicants in the water and feed could markedly shorten this time period by increasing the ascorbic acid requirement.

Halver 1972b and Halver et al. 1975 have suggested that it is possible to use the head kidney ascorbic acid level as an index of ascorbic acid status. Minimum ascorbic acid levels of the head kidney before overt deficiency symptoms were noted were found in our study to be in the range of 30 μg/g. However, the head kidney in young fish is extremely small as compared with the liver and is only difficultly accessible; also, handling stress may markedly decrease the head kidney ascorbate levels (Wedemeyer 1969). Unpublished work in our laboratory has also shown that the dietary and liver levels of ascorbic acid are highly correlated and have a better correlation than diet and head kidney ascorbic acid levels. Therefore, we suggest that the liver is a more appropriate tissue with which to work for the assessment of ascorbic acid status than is the head kidney.

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Effect of Freeze-Up on Major Ion and Nutrient Content of a Prairie Winterkill Lake

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BARICA, J. 1977. Effect of freeze-up on major ion and nutrient content of a prairie winterkill lake. J. Fish. Res. Board Can. 34: 2210–2215.

The reduction of lake volume due to ice formation in a shallow, moderately saline eutrophic prairie lake lacking a permanent surface inflow or outflow accounts for observed increases in specific conductance and concentration of predominant major ions (Mg, Ca, Na, and SO_4). Dissolved inorganic carbon, total ammonia nitrogen, and soluble reactive phosphorus concentrations follow a different pattern; their increase under the ice cover is proportional to the decrease in dissolved oxygen and chlorophyll *a* content.

Key words: winter fish kill, freeze-up, major ions, nutrients, prairie lakes, oxygen depletion, eutrophication

BARICA, J. 1977. Effect of freeze-up on major ion and nutrient content of a prairie winterkill lake. J. Fish. Res. Board Can. 34: 2210–2215.

On attribue les augmentations de conductance spécifique et de concentration d'ions majeurs prédominants (Mg, Ca, Na et SO_4) à la diminution du volume du lac résultant de la formation de glace dans un petit lac de prairie eutrophe, modérément salé, peu profond et dépourvu d'entrée et de sortie de surface permanentes. Le carbone inorganique dissous, l'azote ammoniacal total et les teneurs en phosphore réactif soluble se comportent différemment; leur augmentation sous la couverture de glace est proportionnelle à la diminution du contenu en oxygène dissous et en chlorophylle *a*.

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THE numerous shallow lakes in the prairie region of Canada are regularly under a 6-mo-long ice and snow cover, resulting in lack of exposure of the lake water to atmospheric oxygen, total stagnation due to the absence of surface inflow and outflow, and limited photosynthetic activity. By midwinter these factors eventually cause a dissolved oxygen depletion to near-zero levels and extinction of fish populations (winterkill). Temporary winter oxygen depletion is associated with drastic changes in water chemistry, namely the release of ammonia, CO_2, and H_2S from decomposition processes in the sediments (Green-

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bank 1945; Scidmore 1957; Ahl 1966a; Patriarche and Merna 1970) and is known to have a pronounced effect on the biota in the following ice-free productive season (Nickum 1970; Schindler and Comita 1972). Increases in level of mineral components are also significant during the freeze-up period (Ohle 1953; Ahl 1966b).

In this report an effort is made to analyze factors governing the major mineral components and the nutrients using a simplified freeze-out model, on an example of a typical shallow eutrophic winterkill lake (Lake 885, 50°30′N, 100°10′W Aquaculture Experimental Area of the Freshwater Institute near Erickson, southwestern Manitoba; for chemical and phytoplankton characteristics see Table 1).

Freeze-out model — The physical process of the freeze-out of a shallow landlocked lake can be described by the following assumption: if the total mass of the solute (dissolved solids) in the lake is M, and the import or export of the dissolved material to and from the lake is insignificant (no inflow or outflow, and negligible seepage) for a time interval $t = o$ and $t = x$, this mass is constant: $Mo = Mx$.

Concentrations of the solute per unit volume of the solvent are $Co = Mo/Vo$ and $Cx = Mx/Vx$, respectively, where Vo, Vx are the lake volumes (liquid phase) before and during freeze-up ($t = x$), and $Vx = Vo - Vix$, where Vix is the volume of the ice at time x. Since $Mo = CoVo$ and $Mx = CxVx$

$$Cx/Co = Vo/Vx \qquad (1)$$

This suggests that any decrease in volume (liquid phase) of a lake due to ice formation will be accompanied by an increase in the concentration of solute. This model assumes an insignificant mass of solute in the ice and a complete solubility of the solute in the new volume Vx.

Application of the model to Lake 885 — Lake 885 was monitored on a weekly basis at 1-m intervals at lake center; samples were analyzed for specific conductance, predominant major ions (Ca, Mg, Na, and SO_4), total ammonia (as NH_3-N), orthophosphate (as soluble reactive phosphorus, SRP), CO_2-bicarbonate-carbonate (as dissolved inorganic carbon, DIC), pH, chlorophyll *a* and dissolved oxygen (DO) using methods described by Stainton et al. (1974). Ice thickness, snow cover, and maximum lake depth were recorded at the same intervals (Fig. 1). Actual lake volumes at sampling times were determined by subtracting the volume of ice from

TABLE 1. Lake 885, Erickson-Elphinstone district, southwestern Manitoba. Area 2.4 ha, mean depth 1.9 m. Part. = particulate.

A. Seasonal mean ionic composition (from Barica 1975)

	Specific conductance μmhos/cm	pH	Ca	Mg	Na	K	SO_4	HCO_3	CO_3	Cl	Total ions	Hydrochemical type
			mg/ℓ									
Summer	1395	8.80	38.7	131	93	39	499	380	37	33	1251	SO_4-HCO_3, Mg-Na (intermediate)
Winter	1645	8.22	52.3	182	116	45	644	592	0	47	1678	

B. Seasonal maximum values of nutrients and phytoplankton biomass (from Barica 1975 and Kling 1975)

	NH_3-N	NO_2-N	NO_3-N	Dissolved organic N	Part. N	SRP	Dissolved organic P	Part. P	DIC	DOC	Part. C	Chl *a*	Algal biomass	Predominant algae
	μg/ℓ								mg/ℓ			μg/ℓ	mg/m³ (wet wt)	
Summer	1927	48	691	8195	2604	161	155	169	152	40	155	283	96,000	*Aphanizomenon flos-aquae*
Winter	2993	56	291	6547	435	363	81	57	164	83	36	24	500	Diatoms, Chloroph., Cryptoph.

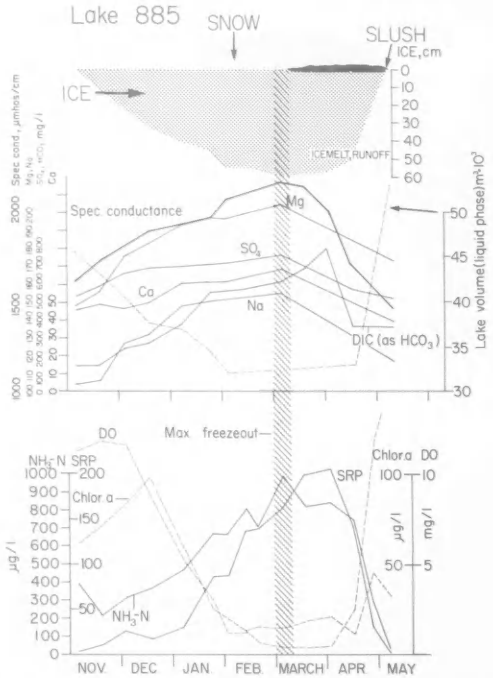

FIG. 1. Changes in major ion and nutrient chemistry of Lake 885 over a winter period, 1974–75.

the October lake volume; they always denote the volume of the liquid phase.

It can be seen that specific conductance, major ions, and the nutrients gradually increase with increasing ice thickness (i.e., decreasing volume of liquid phase), up to the point of the maximum freeze-out in early March. From then on, as a result of the beginning of ice melting, slush formation, and intrusion of runoff water from the drainage basin, they decrease sharply with diminishing ice cover and increasing lake volume (liquid phase). Dissolved oxygen and chlorophyll *a* show a reverse pattern. Their minima occur earlier and persist longer after the maximum freeze-out period. The decrease in lake volume (liquid phase) due to the growing ice volume and the increase of individual ionic components is most pronounced in the period between the freeze-up (November) and maximum freeze-out (March), where no outside effects influence the

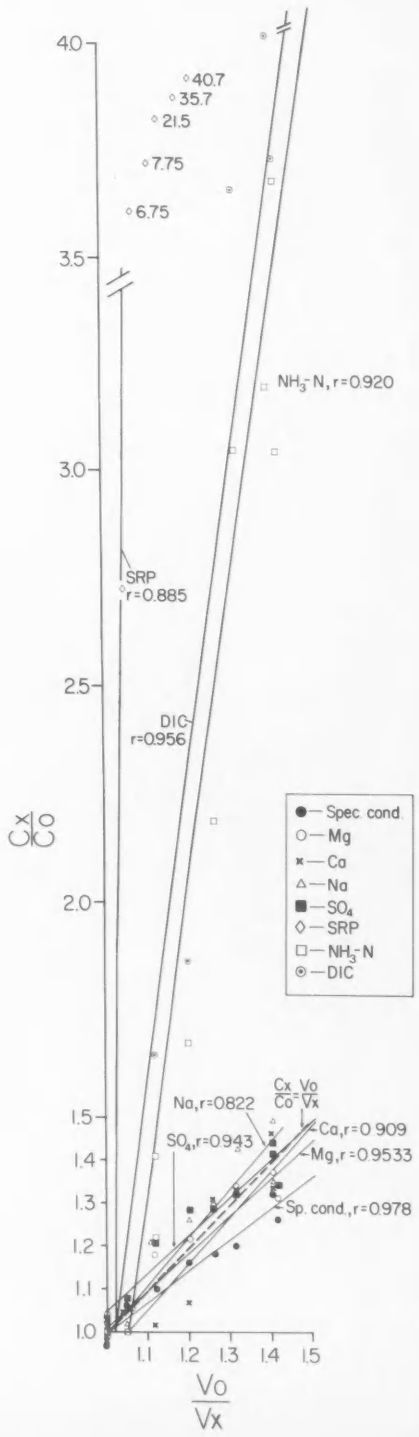

FIG. 2. Regression lines for relative increases in concentration of individual components at sampling times between November 5 and March 5 (from Fig 1) in Lake 885, plotted against the corresponding decreases in lake volume (liquid phase). All correlations significant at the 1% level.

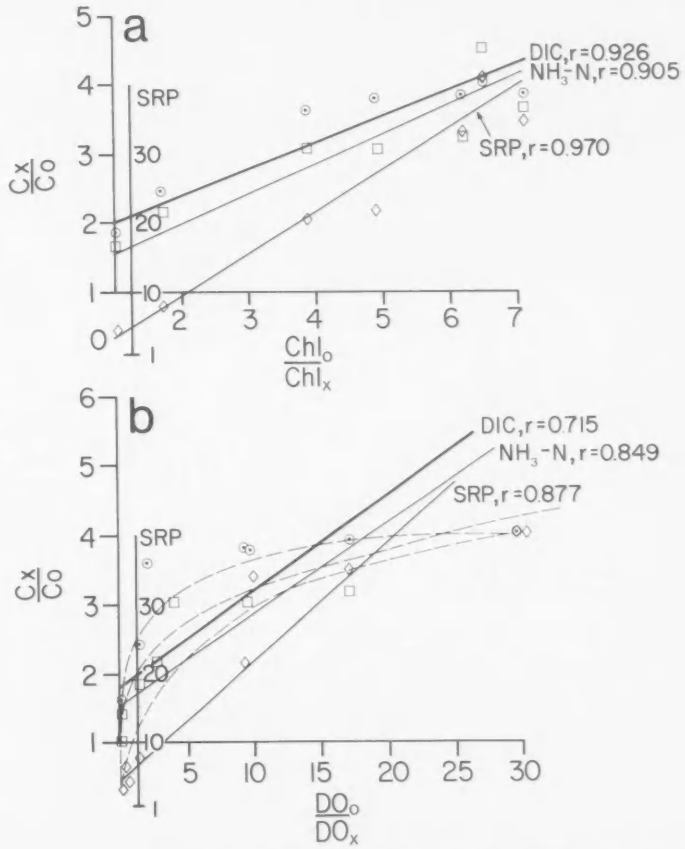

FIG. 3. Correlations between the decrease in chlorophyll *a* (a) and dissolved oxygen (b) and the increase of nutrient concentrations between November 5 and March 5 in Lake 885 (from Fig. 1). SRP and NH_3-N correlations significant at the 1% level. DIC in (b) at 5% level. For data point identification see legend in Fig. 2.

TABLE 2. Relative difference (percent) between the individual concentration ratios *Cx/Co* and the decreases of the lake volume (liquid phase) at individual sampling times.

Component	Mean difference	Max difference	Difference at time of max freeze-out
Spec. cond.	5.3	10.6	5.7
Mg	2.8	7.1	2.1
Ca	5.2	10.8	4.3
Na	7.5	12.7	6.4
SO_4	3.8	8.9	1.4
DIC (as HCO_3)	120.0	185.0	185.0
NH_3-N	99.0	220.0	220.0
SRP	1333.0	2807.0	2807.0

process (basin runoff). This portion only of Fig. 1 will therefore be considered further.

Increases in concentration *Cx/Co* of the individual components for particular sampling times between November 5, 1974 and March 5, 1975 from Fig. 1 were plotted against the corresponding decreases in lake volume (liquid phase) *Vo/Vx* (Fig. 2). Two different groups of regression lines were found.

1. Specific conductance, Ca, Mg, Na, and SO_4 show a distinct grouping of regression lines with slopes similar to each other and to the hypothetical line for equation (1). Analysis of covariance showed that slopes for these five components did not significantly differ from each other and only the line for specific conductance deviated significantly from the hypothetical slope = 1.0. Rodhe

(1949) suggested that correlation between concentration of electrolytes and observed specific conductance was not linear, but represented by a slight curve, with relatively smaller values for higher concentrations of dissolved solids. He attributed this to interionic attraction and other unspecified phenomena. In addition, some precipitation of Ca in a carbonate form, affecting the specific conductance, may also account for the discrepancy. This effect was observed by Otsuki and Wetzel (1974) in a small hardwater lake in Michigan and may occur in our conditions. For the first group (specific conductance, Ca, Mg, $Na.SO_4$) it can therefore be written

$$Cx/Co \text{ (major ions)} = \varphi\ (Vo/Vx) \qquad (2)$$

which means that the increase in ionic concentration is primarily a function of lake volume decrease (liquid phase), and follows the freeze-out model (1).

2. Dissolved inorganic carbon (which in the case of Lake 885 is represented primarily by the HCO_3 ion, i.e. another major ion as well as nutrient) and the total ammonia nitrogen deviate significantly from the theoretical slope by a factor of about 20. Phosphate shows even greater deviation from the hypothetical slope (about 30 times higher).

The mean relative differences between Cx/Co and Vo/Vx (Table 2) for the specific conductance and major ions varied from only 2.8 to 7.5% (a usual analytical error range). For total ammonia, DIC, and SRP the mean differences were more than 10–100 times higher; hence, the nutrients appear to be governed by factors other than physical freeze-out.

It was suggested earlier (Barica 1974) that the nutrient release in small prairie lakes is caused by the decomposition of the dead phytoplankton under anaerobic conditions. To verify this assumption, a similar analysis was applied, using decreases in dissolved oxygen concentrations DOo/DOx and chlorophyll *a* $Chlo/Chlx$ as independent variables instead of Vo/Vx. Increases in DIC, NH_3-N, and SRP (Fig. 3) under ice cover correlated with the reduction of DO and chlorophyll *a*:

$$Cx/Co \text{ (nutr.)} = \varphi\ (Chlo/Chlx),\ DOo/DOx \qquad (3)$$

Thus nutrient concentrations depend on the biological factors associated with the decomposition of accumulated phytoplankton from the previous production season, and not on the physical freeze-out only, as it was in the case of major ions.

It is noteworthy that the distribution of plotted data for the Cx/Co vs DOo/DOx correlation is nonlinear and leads to the origin of the coordinate system 1.0–1.0 (broken line, Fig. 3b). This was expected in all correlations mentioned here, since they all started from the initial ratio 1.0 for Co = Cx at the beginning of the freeze-up. Discrepancy with the Cx/Co vs. $Chlo/Chlx$ correlation (Fig. 3a) occurred because chlorophyll *a* concentrations were still increasing during all of November under the ice (early winter bloom of diatoms and cryptophytes, Kling 1975) and Co equaled Cx at the time when the Vo/Vx ratio was already >1.

Since the oxygen regime in the closed system of an ice-covered prairie lake is governed to a larger extent by the presence or absence of primary production, DOo/Dox is related to $Chlo/Chlx$ and can be eliminated from equation (3). Thus, all concentration increases depend on either of two independent variables (Vo/Vx or $Chlo/Chlx$), or both of them.

I conclude that the winter reduction of lake liquid phase volume due to ice formation affects only the major mineral components, while the nutrients are governed by the winter anoxia and decomposition of algal material.

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Avoidance of Low pH in Selection of Spawning Sites by Brook Trout (*Salvelinus fontinalis*)

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JOHNSON, D. W., AND D. A. WEBSTER. 1977. Avoidance of low pH in selection of spawning sites by brook trout (*Salvelinus fontinalis*). J. Fish. Res. Board Can. 34: 2215–2218.

Brook trout (*Salvelinus fontinalis*) show a strong preference for upwelling water in selection of precise spawning sites. Advantage was taken of this behavior in observing discrimination of pH by 48 breeding females. When artificial aquifers delivered ambient (6.7), alkaline (8.0), and acid water (varied at pH 4.0, 4.5 and 5.0 in three trials), females clearly avoided upwellings of pH 4.0 and 4.5 in preference to alkaline and ambient upwelling. Discrimination was not evident at pH 5.0. As natural springs in the Adirondack area are likely to be alkaline, the strong preferences of brook trout for alkaline upwelling water for breeding sites assures a satisfactory environment through egg and larval stages for at least this environmental condition.

Key Words: behavior, acidity, brook trout, spawning

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Dans le choix d'endroits précis pour la fraie, l'omble de fontaine (*Salvelinus fontinalis*) montre une préférence marquée pour l'eau remontant du fond. Nous avons profité de ce comportement pour observer la préférence de pH par 48 femelles reproductrices. Quand des zones aquifères artificielles fournissent de l'eau du milieu ambiant (6.7), alcaline (8.0) et acide (de pH 4.0, 4.5 et 5.0 en trois essais), les femelles évitent nettement les remontées de pH 4.0 et 4.5 et montrent une préférence pour les remontées d'eau alcaline et ambiante. Au pH 5.0, il n'y a pas évidence de préférence. Comme les sources naturelles de la région des Adirondacks sont probablement alcalines, les fortes préférences de l'omble de fontaine pour les remontées d'eau alcaline dans le choix des frayère assurent un environnement satisfaisant aux œufs et aux stades larvaires, du moins en ce qui concerne ce facteur de l'environnement.

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DISAPPEARANCE of native fish populations in several regions with poorly buffered waters is probably related to acid precipitation. In a survey of high altitude surface waters of the Adirondack Mountains of New York State, Schofield (1976a) concluded that this had a substantial impact on water quality and fish populations during the past two decades. Fish may respond to the environmental change in a variety of ways,

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ranging from alterations in behavior to direct toxicity. Our paper is concerned with the effect of low pH on spawning site selection of brook trout (*Salvelinus fontinalis*), the most important native Adirondack sport fish. Avoidance of areas of acidic water during site selection by spawning fish may enhance egg and fry survival.

Salmonids in Norway were absent in waters where the pH fell below the range 4.6 to 4.8, possibly the result of greater egg and fry susceptibility (Grande 1969). Beamish et al. (1975) reported negative effects of low pH on breeding fish. Ishio (1965) found that fish detected and avoided high and low concentrations of hydrogen ions. Warner et al. (1966) concluded in a review of the literature on bioassays that "quantitative behavioral change is the most sensitive indicator of toxicant-induced change in living systems."

Female brook trout selected areas of upwelling water for spawning sites and the specific site chosen can be controlled under experimental conditions by varying the position of an artificial aquifer (Webster and Eiriksdottir 1976). This aspect of breeding behavior offered a method for observing discrimination of water quality in making this selection.

Materials and methods — Experiments were conducted at a field station in the southwest Adirondacks near Old Forge, New York (long. 75°05′; lat. 48°35′). This facility was provided with a nearly neutral surface water supply from Little Moose Lake (Table 1). The experiment extended between October 15 and November 24, 1974.

An artificial circular spawning tank, 4.25 m diam, was provided with four localized aquifers, or areas of upwelling, located 1.3 m from the walls in each quadrant of the tank. The experimental design was essentially an enlarged version of the facilities and procedure of Webster and Eiricksdottir (1976). Aquifers, made of inverted perforated pie tins (17.5 cm diam), and connecting tubing were covered with a 20-cm layer of 2–4 cm gravel; the bottom above the aquifer was veneered with finer gravel over an area 90 cm diam to help detect redd construction. A water level of about 30 cm was maintained above the gravel substrate. Uniform flows were maintained between aquifers during trials, but varied from 11.4 to 19.0 ℓ/h between trials. Delivery to upwellings was by gravity through ID 0.108-cm tubing from 114-ℓ capacity reservoirs. About 11.4 ℓ/min of fresh lake water were added to the pool near the peripheral discharge to maintain the ambient tank water quality. The pool was lighted by daylight entering on three sides of the room. Preliminary trials with lake water upwelling concurrently at all four aquifers did not suggest position effect in selection of spawning areas at the four sites.

Experimental trials provided a choice of water quality at each of three positions: acidified (pH 4.0, 4.5, 5.0), alkaline (pH 8.0), and natural lake water (pH 6.7). The fourth position had no flow. Each active upwelling directly influenced about 2.2% of the spawning pool substrate, judging from the pattern of Rodamine B dye injected into the water supply. Dye-marked water rose slowly in a column 16–20 cm above the aquifer before dissipation. Each treatment was randomly varied to appear 4 times at each upwelling position using 16 individual trout in trials at pH 4.0, 4.5, and 5.0, for a total of 48 observations.

TABLE 1. Analysis of natural and acidified water from Little Moose Lake, Old Forge, N.Y. All values except pH and conductivity expressed as milligrams per litre.

Item	Acidified	Natural
Calcium	3.3	2.6
Magnesium	.58	.48
Sodium	.78	.56
Potassium	.36	.30
Sulfate	4.60	1.80
Ammonium	.021	.013
Nitrate	.28	.20
Chloride	.30	.22
Bicarbonate	< .50	4.21
Conductivity μmhos/cm @ 25°C	63.25	31.75
pH	4.0	6.75

Sulfuric acid (6 N) was used to acidify the field station water to pH 4.0 (Table 1). After 16 individual runs at pH 4.0, the acid level was reduced to pH 4.5, and finally to a pH of 5.0. The pH was estimated from conductivity, utilizing calibration curves prepared by adjusting samples of the water supply with H_2SO_4 to known pH and conductivity levels. Every individual mixture was checked by Accumet Model 420 Digital pH/Ion Meter, a procedure permitting maintenance of the exact nominal pH. The pH of natural spring water was determined after 30-min aeration of samples to equilibrate CO_2 levels. Raw spring water samples usually had pH values of about 6.7 because of supersaturation with CO_2. Addition of reagent grade sodium bicarbonate to the lake water created an alkaline upwelling of pH 8.0, with variation not exceeding pH 8.0, ±0.1 pH units for all trials.

Water in containers servicing the aquifers was uniform and at room temperature of about 10°C. Spawning pool temperatures averaged 7, 8, and 7°C at the three test pH levels, respectively.

Gravid or nearly gravid female brook trout varying from 18 to 41 cm total length were trap netted from natural populations in four nearby lakes. Segregated sexes were held in the same water supply as the test facility but at the declining lake temperature regime of 9.6–3.4°C. Gravid females were used as test fish and generally only one at a time to avoid competition for spawning sites. Two males were present, however, and these were reused so long as they maintained strong courtship behavior. Males with this experience began courtship much sooner than new fish, appreciably decreasing time for females to begin searching for spawning sites. Initiation of redd construction ranged in time from 4½ to 48 h,

TABLE 2. Choice of redd site by 48 individual brook trout females in relation to pH of upwelling water in an artificial pool of ambient water at pH 6.7.

Upwelling water quality	Test pH 4.0 pH	4.0 No. fish	4.5 pH	4.5 No. fish	5.0 pH	5.0 No. fish	Total
Alkaline	8.0	9	8.0	8	8.0	6	23
Ambient	6.7	6	6.7	6	6.7	5	17
Acid	4.0	0	4.5	1	5.0	3	4
No upwelling	—	1	—	1	—	2	4

averaging 16. Criteria for redd site selection was the same as described by Webster and Eiriksdottir (1976): the initial pit constructed constituted the site of choice and trials were generally terminated once this commitment was made. The gravel bottom was raked smooth between trials.

Results — Female brook trout clearly avoided areas of upwelling at pH 4.0 and 4.5 (Table 2). Of the 32 females, only one initiated redd construction in the quadrant containing a strongly acid aquifer, and this pit was not located directly over the upwelling. In contrast, 29 females built pits closely asociated with the zone of upwelling water at pH 6.7 (ambient) or 8.0. Two females used the quadrant without upwelling. When upwelling water was at pH 5.0 there was no clear choice as the initial selection sites were distributed in all four quadrants and close to an expected frequency distribution of 4:4:4:4. For the overall experiment, expected random use of each quadrant was $48/4 = 12$, and the observed frequencies of 16:12:8:12 justified the conclusion that there was no significant position effect in selection of redd sites.

Ancillary manipulation of upwelling water quality provided additional suggestions on behavioral interactions of female brook trout in reacting to shifts in this variable. The pH of upwelling water was changed from 6.7 or 8.0 to 4.0 after eight females had started definitive pit construction. Four fish in initial stages of redd construction eventually abandoned the site and spawned elsewhere. Significantly, four females that had spawned and were in either postspawning sweep or pit-covering stages of behavior did not desert the upwelling of choice, but continued the normal behavioral pattern until the eggs were covered.

Discussion — Under the conditions of this experiment, female brook trout failed to show clear cut avoidance of upwelling water above pH 4.5. Further refinements in testing discrimination of water quality require offering smaller changes in pH of upwelling water above and below pH 5. Conditions of highly acid upwelling spring water are not likely to be often encountered in the Adirondack Mountains, even though surface waters are usually more or less acid (Schofield 1965). As examples, relevant parameters in five "boiling" springs located in the vicinity of the study area were as follows:

Spring	pH	Alkalinity μeq/ℓ	Conductivity μmhos/cm @ 25°C
Green Lake Camp	6.72	50	33.5
Pico	7.39	232	50.2
Hatchery Camp	7.56	349	61.8
Joy Tract	7.81	400	63.3
Jones Lake	8.70	262	47.1

In Jones Lake, surface waters declined from pH 6.9 to 4.8 between 1960 and 1976, while water chemistry of the shoreline spring remained relatively constant. Currently, surface waters in the southwest Adirondacks commonly have pH values of 4 or 5, although large seasonal variations may be encountered (Schofield 1976b).

This experiment indicated that the strong preference shown by female brook trout for alkaline upwellings and the probably alkaline nature of groundwater available would insure deposition of eggs in a pH environment favorable for development of eggs and larvae. The fate of free-swimming fry in highly acid surface water outside the influence of springs is another consideration (Trojnar 1977). Brown trout fry were nearly absent from a highly acid (pH 4.8) tributary of a Norwegian lake, formerly the principal spawning area, but were abundant in a less acid smaller tributary with a pH of 6.2 (Leivestad et al. 1976). Their observations on success of natural spawning were substantiated by survival of artificially planted eggs.

Thresholds of acidity below which normal spawning behavior is inhibited are unknown; in preliminary observations ancillary to our study, six pairs of breeding fish subjected to pH 4.1–4.3 ceased normal breeding behavior after 48 h and became distressed after 72 h. When pH was returned to 6.7 (that of ambient pool water) two of the females completed spawning. Alkaline upwellings in an otherwise acid medium could also modify thresholds of breeding behavior.

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A Titration Apparatus for the Determination of Dissolved Oxygen in Seawater

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A novel titration apparatus for the Carritt/Carpenter modification of the Winkler method for determining dissolved oxygen in seawater is described. Routine quantitative analyses for oxygen are made more reliable and more convenient by reducing the human factor.

Key words: analytical techniques, oxygen measurement, seawater, oceanographic expeditions

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Les auteurs décrivent un nouvel appareil de titrage applicable à la modification Carritt/Carpenter de la méthode de Winkler de détermination d'oxygène dissous dans l'eau de mer. Une diminution du facteur humain rend les dosages quantitatifs routiniers d'oxygène plus fiables et plus faciles.

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RECENTLY Cunningham et al. (1976) described a device for dispensing the reagents used in the Carritt and Carpenter (1966) modification of the classical Winkler method for the determination of dissolved oxygen in seawater. In this note we describe an apparatus for performing the analyses, which like the dispenser, has evolved over the past several years in an effort to eliminate the "human factor" from the analyses and thereby improve the quality of analytical data collected during oceanographic expeditions.

The apparatus (Fig. 1) consists of the following components: a Labindustries Repipet (1 ml)

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(1) used to dispense the sulfuric acid, which initiates a series of chemical reactions that ultimately generate an amount of iodine complex equivalent to the amount of oxygen originally present in the seawater sample; a Cornwall pipetting syringe outfit (2) (consisting of a Cornwall Luer-Lok Tip syringe, a metal holder and a filling outfit, which includes an assembly of automatic two-way valves, a spherical metal sinker, and connecting tubing) to dispense the starch suspension used as indicator; square glass bottles (3) (Labindustries) for reagents; a Metrohm Dosimat digital buret (10 ml) (4) for adding the sodium thiosulfate used as titrant; and a Thermolyne Stir-Light (Model No. SL-7225) (5) illuminated magnetic stirrer. (Although components of other manufacture would probably serve equally well, the above-mentioned items have proven trustworthy and consequently we have no reservation in recommending them. Total cost of components is approximately $600 at 1977 prices.) The surface of the stirrer is covered with a sheet of translucent white Nalgene (6) which has an opening cut to fit the bottom of the oxygen sample bottle (7), a 125-ml calibrated iodine flask with a custom stopper (8) that displaces sufficient water so that the titration can be carried out in the sample bottle as described by Carritt and Carpenter (1966). The Nalgene sheet ensures that the bottle is positioned directly over the magnet and light source of the stirrer. While seemingly a trivial detail, this is a very important aspect of the apparatus. Not only does it ensure uniform and repeatable conditions of stirring so that the rates of mixing and color change are uniform from sample to sample, but more importantly, it creates conditions of illumination that greatly facilitate the visual detection of the endpoint of the titration. While the Nalgene surface provides a background of uniformly diffused light of reasonably constant intensity, the full intensity of the light passes through the opening and into the flask so that considerably brighter light emanates from within the sample itself. Under these conditions, an abrupt flash is emitted from the solution simultaneously with the complete disappearance of the blue color at the equivalence point of the titration. The components are arranged for the convenience of the analyst, and to prevent breakage at sea, they are securely mounted on a 45 × 60-cm sheet of 1.9-cm plywood. This is also covered with Nalgene sheet to make cleaning easier and to eliminate sharp contrasts in the illumination of the work surface. The entire assembly is readily transported as a unit and can be firmly attached to the workbench on the ship to avoid spilling the reagents or breaking the components during rough weather.

FIG. 1. Titration assembly for oxygen analyses on oceanographic cruises. See text for explanation of numbers.

All reagents are dispensed from preset dispensers and there is virtually no chance of error from adding too much, too little, or the wrong reagent and there is no danger of cross-contaminating reagent stocks. Since pipetting and transferring of subsamples have been eliminated, so have the errors inherent in these procedures, both of which are onerous tasks to perform at sea. Digital burets of the Dosimat type are vastly superior to conventional types for routine analyses and are particularly useful for work at sea. Because the rate of delivery of titrant is controlled electrically, the reagent can be added in very precisely controlled increments and the titration can be stopped exactly at the endpoint. This avoids a major frustration with stopcock burets at sea; that is, just as the last fractional drop of titrant is being added before the endpoint, the ship will give an unexpected lurch throwing the analyst off balance and resulting in overshooting the endpoint. In addition, the digital readout is much more convenient than reading graduations on a glass tube and attempting to interpolate them. The problem of reading a meniscus is eliminated. As an additional desirable feature, the Metrohm Dosimat has an electrically operated refilling and automatic rezeroing mechanism. We have found these burets to be completely reliable and trouble-free even under the most difficult conditions at sea.

By far the most desirable feature, however, is

the ease and sensitivity with which the endpoints are detected under the conditions of illumination provided by our equipment. As a consequence, it is not only a simple matter for an experienced analyst to perform the analyses, but it is also a comparatively easy matter to train new personnel and even those with little, or no, previous experience soon produce reliable data. With the measurements made at sea, overall reproducibilities, including sampling and analysis, of 0.01 ml/ℓ of dissolved oxygen are readily attained for replicate samples and can be achieved whether the analyses are performed by the same or by different analysts. This frequently simplifies the assignment of work tasks and the logistics of carrying out oceanographic studies at sea.

CARRITT, D. E., AND J. H. CARPENTER. 1966. Comparison and evaluation of currently employed modifications of the Winkler method for determining dissolved oxygen in seawater. A NASCO Report. J. Mar. Res. 24: 268–318.

CUNNINGHAM, C. C., C. D. W. CONRAD, J. D. MOFFATT, AND E. M. LEVY. 1976. Improved reagent dispenser for the determination of dissolved oxygen in sea water. J. Fish. Res. Board Can. 33: 2076–2078.

LETTERS/LETTRES

Fish Catches and Environment

Comments

The recent paper by Sutcliffe et al. (J. Fish. Res. Board Can., Vol. 34: 19–30) raises a couple of important issues. The first concerns the statistical treatment. The authors touch on the difficulties in assessing the significance of the observed correlations caused by the selection of environmental signal, the month, the smoothing period, and lag time. Combining these with the perennial difficulties in time series, especially when the variables exhibit simple trends (e.g. redfish or striped bass) or one or two marked peaks or troughs (e.g. alewife, menhaden or yellowtail flounder), these must raise considerable doubts on how definitely the relations have been established, and how closely catches are indeed determined by the chosen environmental factors.

The other point is the continuation in this paper of the unfortunate dichotomy in approach between considering catches or fish abundance being determined entirely by fishing, or entirely by environmental factors. The former approach could be defended, at least in the past, by the need to advise governments and others on the desirable level of fishing. If the advice is to be timely, and thus effective, the first round of analysis of the effects may have to ignore possible changes in the environment, but this can be regarded as acceptable only as a first approximation. It seems even less acceptable, when considering the effects of environmental factors on fish stocks, to fail to consider at the same time the effects of fishing. In the region (ICNAF subarea 5) examined by the authors, there have been extensive studies of the effects of fishing. These have shown that fishing has been having a dominant effect on many of the stocks, and that many of the observed changes in catch can be explained by changes in the pattern of fishing. Admittedly many of the studies are in reports of ICNAF working parties and similar material, rather than in the conventional published literature, but these reports can be obtained with little difficulty through ICNAF, or national laboratories. The logical approach to examining the effect of environmental factors would therefore seem to be the examination of the residual variations, once the expected effects of fishing, as predicted by these studies, have been taken into account. This could simply involve correlating deviations between expected and observed catches with environmental signals (with suitable lags and averages) and would have the statistical disadvantages already noted. Enough is known of many of the stocks to suggest procedures that are likely to be more closely related to the actual biological processes. For example, the variation in haddock catches, over and above the direct effect of fishing, seems to be largely due to fluctuations in year-class strength. Good data on year-class strength exist, so that it would be possible to look directly at possible correlations between year-class strength and environment. This seems more likely to provide insight on what factors, other than the fishery, cause changes in the stock abundance, though again caution is needed against statistical traps and there is the need to consider simultaneously with the environment the possible effects of different levels of stock abundance, especially in the last decade when the stock level has been very low.

While it is highly desirable that a closer examination of the effects of environment be made than has often been the case in the past — and FAO hopes in the future to do more to help stimulate this examination – this has to be done in close collaboration with the studies of the direct effect of fishing. — J. A. Gulland, *Food and Agriculture Organization of the United Nations, Rome, Italy.*

Reply

It is, of course, impossible to disagree with Mr Gulland's two main points: (1) one should be cautious concerning results obtained by correlation analysis (or any analysis for that matter) and (2) the research could have been done in some other fashion. Any research is governed or limited by the investigator's inclinations, abilities, and the tools and time at hand; ours is certainly no exception.

Concerning the first point, when one is confronted by a statistical result, the dilemma is not only in rejecting the false or spurious but equally in accepting what may be true or leading to

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insight. Our answer to this was to devise what we thought to be a more conservative and more rigorous test for significance (details in Sutcliffe et al. 1976). We do not pretend this is the best or final answer but perhaps it is a step in the right direction. We do not wish to be on record as saying that catches are necessarily *determined* by the environmental factors we considered simply because of similarity with catch records; other signals may vary in concert, and temperature may be only an indicator.

We would like to make the following points:

1) The lag time found for most species was not greatly different from mean age at commercial size – a finding in agreement with the general belief that survival is related to events in the 1st year of life.

2) In general, negative correlations were found for more northern species and positive for southerly ones.

3) Smoothing is an acceptable statistical filter for examining longer term or low frequency events, but Table 4 where no smoothing was used should be noted.

In connection with this we would like to draw attention to a forthcoming paper on an independent investigation (Dow 1977). In this most of the same species were used, together with simple annual means of a single signal (Boothbay Harbor temperatures) with no smoothing, and much the same conclusions were reached.

Concerning the second point, we must apologize if we have left the impression that environmentally associated fluctuations should be considered to the exclusion of other equally (or more) important factors such as fishing. This is clearly a limited point of view and it was not our intention. We urge only that the environment be considered as well and look forward to results from the FAO studies mentioned by Mr Gulland.

We also have been giving closer attention to the combination of environment and effects of fishing and hope to submit some results soon. — W. H. SUTCLIFFE JR., K. DRINKWATER, AND B. S. MUIR, *Department of the Environment, Fisheries and Marine Service, Marine Ecology Laboratory, Bedford Institute of Oceanography, Dartmouth, N.S. B2Y 4A2.*

DOW, R. L. 1977. Effects of climatic cycles on the relative abundance and availability of commercial marine and estuarine species. J. Cons. Cons. Int. Explor. Mer 37(3). (In press).

SUTCLIFFE, W. H. JR., R. H. LOUCKS, AND K. F. DRINKWATER. 1976. Coastal circulation and physical oceanography of the Scotian shelf and the Gulf of Maine. J. Fish. Res. Board Can. 33: 98–115.

Aquatic Environmental Quality: Problems and Proposals

COMMENTS

The review (Harvey 1976) contains many valuable observations, conclusions, and recommendations, but also some ranging from disputable to incorrect. The author limits the role of chemistry in aquatic environmental quality to "water chemistry" (meaning the determination of inorganic and organic constituents of natural waters), and most of the discussion on individual pollutants is devoted only to mercury and PCBs (incidentally, the abbreviation stands for polychlorinated *biphenyls* not *biphenols*). These limitations do not do justice to the actual function of chemistry in environmental research.

It is true that many constituents of natural waters are measured much too frequently and relatively less attention is given to other components, more important for water quality. For example, water hardness was measured in 52 and dissolved oxygen in 28 samples per year in a routine monitoring program. It is important "to identify ways to reduce the number of samples being analyzed." The best way to achieve this is to provide a competent scientific leadership, involving environmental chemists. Chemistry is not just a service for environmental programs and should participate in leading these to the same extent as biology.

The suggestion "to reduce the country-wide efforts expended on new methods" is wrong. Chemists are not developing new methods in a display "of displacement behavior: an opportunity for publication and research in what is an otherwise dull routine." For various reasons (accuracy, precision, speed, cost, lack of speciation, etc.), many of the currently used methods are not satisfactory. In addition, methods are not available for many contaminants or potential contaminants, and the presence of those not anticipated may not be detectable by existing methods.

The author suggests "to better relate water chemistry data ... to aquatic organisms and their water quality requirements" and, at the same time, recommends to reduce the effort "devoted to ... fish physiology/toxicology." It is impossible to accommodate simultaneously both suggestions. On the contrary, the second effort should be expanded to cover a wide variety of aquatic flora and fauna.

Early warning systems are discussed too briefly

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for their importance. The statement that "fish were useless as indicators" in the case of mercury pollution is incorrect. High levels of mercury in aquatic fauna were among the first indicators of mercury pollution. What the quoted statement really means is that purely ecological monitoring of the environment is not a sufficiently sensitive early warning system. Even if it were, other disciplines would have to assist to identify the causes. PCBs would not have been identified as pollutants by ecological monitoring alone.

Persistent compounds can be detected relatively easily by analyzing biological samples, provided the chemists have enough time and support to develop methods. Nonpersistent poisons are much more difficult to detect, and physiological and biochemical parameters, such as enzyme activity, must be used in addition to a purely chemical screening.

The author's additional suggestions about the present early warning systems, "identify all hazardous materials used in our society" and "to monitor some substances in the environment," are not different from the current approach and contradict his recommendation not to spend so much time developing new chemical methods and conducting toxicological research.

The outline of man's activities leaves out chemical industry and the discussed activities are not well balanced in respect to their relative importance. For example, very little attention is given to agricultural chemicals, whereas research needs in respect to forestry are discussed in detail.

Most of the comments dealt with "Monitoring" recommendations 5–7. Some of the "Research In General" recommendations are general indeed and 1–5 could be reduced into one, saying that there is a need for more research and innovation. Recommendations 6–10 are good. A closer cooperation of different disciplines in solving environmental problems cannot be overemphasized. More than putting people in one building is needed to achieve it, and the participation in cooperative interdisciplinary projects may be the best approach.

Industrial wastes enter the review for the first time under the "By Economic Sector" recommendations. Again, a considerable input from environmental chemistry is needed. Many of the recommendations in this section are for Development rather than Research. See for example "research on alternate treatment systems ... ," treatment of emissions and effluents from power generators, mining industry, "major research into the technology of sulfur dioxide."

Mercury is not the only "most horrifying material used in the industry." There are many other toxic chemicals.

In conclusion, Harvey devoted a lot of time and effort to reviewing the problems of aquatic environmental quality in Canada, but the review is only a starting point for a discussion of these problems before formulating a definitive policy. Environmental experts with different backgrounds should be involved in this discussion. — V. Zitko, *Department of Fisheries and the Environment, Fisheries and Marine Service, Biological Station, St. Andrews, N.B. E0G 2X0.*

Harvey, H. H. 1976. Aquatic environmental quality: problems and proposals. J. Fish. Res. Board Can. 33: 2634–2670.

Comments

As mentioned by the author, this article (Harvey 1976) was commissioned by the Fisheries Research Board as a background document for an overview of government policy in relation to aquatic environmental quality. As such it deserves to be taken seriously. Its shortcomings in understanding the need for more effective input from organic chemists, and the important role that such scientists must play, has to be discussed. It is with this in mind, and through no desire to belittle the efforts of the author in his review of the problems, that the following comments are offered. Let us begin by considering the reasons why the Fisheries and Marine Service should be placing more emphasis on organic chemistry as a means to achieving its objectives.

A. There is an obvious need to search for the presently unrecognized chemical stress factors in the Canadian environment, especially those organochlorine compounds entering receiving waters from municipal or industrial sewers. Such dangerous compounds may derive from the chemical industry, from the bleaching of wood pulp, from the household use of chemical formulations, or from the disinfection of municipal sewage in primary treatment plants.

B. As a corollary of the above, there is a need to establish which compounds are dangerous environmentally through bioaccumulation in the food chain or which can cause tainting of the flesh of resident fish populations near pulpmill or sewage outfalls. Such information is presumably vitally needed under the terms of the Fisheries Act, the new Ocean Dumping Control Act, and the Environmental Contaminants Act.

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C. It is essential, and this cannot be overemphasized, that an organic chemist should be a member of any team that has been assigned the task of finding out which chemicals in a complex organic effluent are environmentally dangerous. If one knows what these compounds are, strategies can be developed to prevent their discharge or it may even be necessary to change to a different type of effluent treatment. It makes no scientific sense to continue to perform bioassays on complex organic wastes, as in the past, without having a knowledge of the chemical composition of the effluent at the time the bioassay data were gathered. This subject is covered in a review paper (Lee 1973) with which policy makers in the Fisheries and Marine Service should be familiar.

D. Sanitary engineers should also work in conjunction with organic chemists to discover how best to degrade or remove certain kinds of persistent waste products, for example nonionic detergents. Is there a danger that the metabolites may be more toxic or more bioaccumulable than the original products?

E. The Environmental Protection Service has no research mandate in Canada which, in this writer's opinion, is a mistake that was not made in the United States, when the Environment Protection Agency was established. E.P.S. should have organic chemists on staff or available to it in other services of the Department of Fisheries and the Environment to find out why certain industrial or municipal waste samples from point source discharges are toxic to aquatic organisms. Now with the extra burden of demands arising from recent legislation — especially the Environment Contaminants Act — the few chemists on staff will be entirely unable to meet the potential demands. On the west coast the Canadian Forestry Service has three organic chemists, Inland Waters Directorate one, Ocean and Aquatic Sciences one, and Fisheries Service three of whom only one is in a laboratory with a defined research mandate. When methods are needed — and needed they will be — to analyze the multitude of organic compounds in the wastes discharged by today's society, who will be available and who will have the manpower and the financial resources to come up with the needed answers? The instrumentation needed is in itself costly and requires regular maintenance expenditures. However, it is not necessary for every laboratory to have mass spectrometers or nuclear magnetic resonance spectrometers and one well-equipped facility could take care of the requirements of all the interested government departments on the Pacific coast.

The article in question does not identify or address itself to these problems and does not distinguish between inorganic and organic chemistry. Thus it may well be true that some agencies within the Department of Fisheries and the Environment are obsessed with measuring heavy metals, nutrients, grain size of sediments, and other routine parameters on field samples. These tests are usually easy to perform, the things being sought are discrete and usually easily identified. On the other hand, organic compounds are not so easily analyzed and the methodology employed for cleanup, the strategy involved in chromatographic separations, and mass spectrometric identifications and so on is endless in its variety. Moreover, of the "one million-plus" chemicals that may be entering Canadian waters, probably 90% would be organic compounds because of the infinite variety of chemical combinations in which carbon may be found.

The article refers to problems of possible overlap in environmental studies between scientists employed by the federal government and by the provinces. This may be true in terms of the development and application of regulations or guidelines. It certainly is not true in terms of innovative research in British Columbia. This province does its own chemical monitoring, but it does not attempt to cover any of the true research activities of the organic chemist listed in A through E and makes use of information generated by federal or university scientists. It is highly doubtful that any of the other provinces are competing in this regard.

A great frustration for environmental chemists in the federal service concerns their inability to make use of the large pool of talent available in the university community. This would be possible if money were made available within suitable constraints, so that research contracts could be let for specific studies in university chemistry departments. Such work should cover areas that have been outlined by federal scientists as giving rise to real problems. However, it often is true that constraints of manpower, equipment, and expertise would indicate that further work on the problem could be done more profitably in the universities under contract. Unfortunately, research scientists in the Fisheries and Marine Service whose work may have outlined such problem areas have virtually no access to such funding for the awarding of contracts and their recommendations for expenditures for such contracts through available channels seem to bear little weight.

The author was incorrect to conclude that sufficient research work is now going on in relation

to point source discharges. From what has already been said in this critique, clearly very little organic chemical involvement has occurred in this country relating the toxic effects of chlorinated sewage to its chemical composition. The same is true of the exceedingly complex mixtures of organochlorine compounds formed by the bleaching of wood pulp. Another example is the unknown nature of the chemicals responsible for taste and odor problems in fish within the zone of influence of either pulpmill or sewage outfalls.

Some areas of serious concern where organic chemists could make an important contribution are currently almost being ignored in Canada. On the west coast, which will imminently be exposed to the hazards of massive oil shipments through treacherous waters once the Alaskan oil pipeline begins to function, only one federal scientist is at work in the Marine Service and none in the Fisheries Service. An excellent symposium was held in Seattle, Washington, in November 1976 on the "Fate and Effects of Petroleum Hydrocarbons in Marine Ecosystems and Organisms." Of the 71 papers presented, only three were authored by Canadians — all from the east coast. No research is being conducted on the Pacific coast regarding the levels of pesticides in fish stocks, or lower trophic levels, although the Fish Inspection Laboratory may be routinely examining samples. In this regard, the author correctly identified as one of the problems the keeping of such information secret.

Finally, one has to agree that there is an obsession with the collection of data in water chemistry and very little interpretation being made based on that data. Sometimes the methods in use are so suspect that the data are virtually useless in any sense that would involve the explanation of biological phenomena. An example of this would be measurements of resin acids made via the colorimetric method, still currently in use by the pulp and paper industry. Here it has been found that certain common resin acids do not give a color with the reagents. Another example is the measurement of "tannins and lignins," often called for in permits granted for industrial discharges. In this case, the significant parameter is color which can be measured directly. This writer knows of no direct toxic effects of either tannins or lignins on aquatic organisms. Color, however, prevents penetration of sunlight into the water column and can interfere with the growth of phytoplankton.

In conclusion, clearly the job of protecting water quality in a country as large and diverse as Canada is a big one and requires the pooling of experience from research people with training in many scientific disciplines. The author of the review revealed his particular bias when he mentioned that not enough effort was being expended on ecosystem approaches and population dynamics, whereas this writer leans rather heavily towards the use of scientists with training in organic chemistry to improve the balance of the system, which in the past has been too heavily oriented towards the biological sciences. Hopefully all points of view will be heard before important and irretrievable decisions are made on future approaches to study and protect the quality of the aquatic environment in Canada. The organic chemist has a vital role in gaining an understanding of the effect of pollutants on aquatic organisms and ecosystems. Certainly his capabilities should be utilized in cooperation with biologists to resolve some of the unsolved problems in controlling pollution of the aquatic environment and protection of the living resources. — I. H. ROGERS, *Department of Fisheries and the Environment, Fisheries and Marine Service, Pacific Environment Institute, 4160 Marine Dr., West Vancouver, B.C. V7V 1N6.*

HARVEY, H. H. 1976. Aquatic environmental quality: problems and proposals. J. Fish. Res. Board Can. 33: 2634–2670.

LEE, G. F. 1973. Chemical aspects of bioassay techniques for establishing water quality criteria. Water Res. 7: 1525–1546.

REPLY — You Can't Really Get There from Here

Protection of the aquatic environment in Canada has several weaknesses. There is excessive dependence on a single strategy, that of chemical identification, toxicity measurements, setting standards, regulations, seeking compliance, etc. The federal effort in maintaining the quality of the aquatic environment is large and cannot be expected to be increased in the near future. The resolve to use the information available is weak. Protection of the environment could best be described as a weak political position maintained by strong troops. It seems appropriate that we look now for alternative, less costly strategies for protecting the environment.

The Zitko–Rogers comments make a strong plea for more federal initiative/effort in the organic chemistry component of the sequence: contaminant identification, toxicity testing, criteria setting, promulgating regulations, enforcement, etc. I accept that this is an important strategy in protecting the environment. What I

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was objecting to so inadequately in my background study for the Fisheries Research Board (Harvey 1976) was that this has come to be regarded as the *only* strategy. I was objecting also to every environmental problem being seen as a federal responsibility with the endless pleading for more money and man-years. I was trying to indicate that the weakness of our environmental protection was not due primarily to the shortcomings of our data base, but rather to our lack of *resolve* to use what we know. Environmental protection could best be described as a weak position maintained by strong troops. Perhaps a democracy is obliged to operate in such a way — I don't know. I do know that the cost of protecting the environment in this way is high and I believe that we should look carefully at alternative strategies. I will expand on these four points.

I acknowledge many of the weaknesses identified by the critics, for example the failure to make clear the separation of water chemistry into organic and inorganic components. My concern was with the extraordinary amount of effort, federally and in each province, devoted to the development of new methods for the routine analysis of ions, the number of such analyses performed, and the development of the many data bases for storing this information. I am not convinced that this duplication of effort was the best use of our national environmetal-protection resources. I think there is little point in acknowledging spelling errors, putting phrases back into context, or in explaining apparent ambiguities.

Part of the solution or part of the problem? — While writing this I have before me a permit issued to a chemical company on the south side of Lake Ontario, authorizing the dumping of up to 950 lb a day of chlorinated hydrocarbons into Lake Ontario. (I have chosen this example from (just) outside Canadian jurisdiction in the hope that I can minimize the likelihood of offending some new group of people and hence be obliged to write another rebuttal). These chlorinated hydrocarbons are unspecified as to composition, concentration, life expectancy, degradation products, or synergistic actions. (I am told, but have not verified, that the company in question produces only half that amount of such waste, but accepts the remainder from other industries for the purpose of discharging it through their permit). We could spend easily the rest of our natural lives analyzing and doing toxicity tests on this witches brew. Eventually we or our descendants could write a set of criteria for such a mix. That is one strategy. There are other strategies. One is to write a permit for such a discharge that reads like this: *"You can't put that damned stuff in the water until you demonstrate that it is harmless."* Faced with the absurdity of such a demonstration, the company would be obliged to search for alternative development strategies. But when government accepts the responsibility for the demonstration of harm, through chemical identification, toxicity, etc., it may delay or preclude a solution. Government may cease to be part of the solution and become instead part of the problem.

A classic example of the constipation of such a strategy is that of Reserve Mining dumping daily 67,000 tons of tailings into Lake Superior. After 20 years, half the life of the mine, can we really pretend that a control strategy is working? I am not so naive as to imagine that the Reserve Mining problem has a simple cause and solution; there is a plethora of extenuating circumstances/excuses. But we should all forego our naivety in thinking that such excuses protect the environment; they don't, they merely protect the people involved.

Parenthetically, it is interesting to note the difference in strategy between ocean and freshwater dumping. The Ocean Dumping Control Act identified categories of substances under two schedules: Schedule I substances are prohibited, Schedule II substances require special care (Guide 1975). At the top of the list under Schedule I are the organohalogen compounds. There is no implication that we need to know the toxicity of each and every one of these; ocean dumping is simply banned. Can anyone explain to the public why chlorinated hydrocarbons because they are so toxic are banned from ocean disposal, and at the same time can be so nontoxic that massive freshwater disposal is permitted?

Has anybody seen my mandate? — The commonest plea one hears in the federal service is for more resources: more man-years, more dollars. The need for more is justified on the basis of the myriad of problems which are identified and need to be solved, and almost always needs are perceived as a federal responsibility requiring direct federal application of human and financial resources.

Frequently these perceived needs extend beyond any existing mandate. The shock waves that are passing through the Department of Fisheries and the Environment (DFE) currently are in large part due to this inharmony between mandate and activity. The federal forestry role is an obvious example: what was once a department reduced to half a department, then to a service, then a service within a service, then a directorate with an overall one-third reduction in manpower. The same kinds of questions are being asked of other

services in DFE, for example does the department need one of the largest Planning and Finance units in government, with 757 man-years identified as administrative program, 1977–78. What is the federal mandate in lands? There is no mandate for a federal role in interprovincial waters or for coastal zone planning, but we behave as if there were.

Becoming more specific, why should federal fisheries agencies accept the responsibility for determining the toxicity of chlorinated sewage? Indeed, they have gone even further and are actively researching alternative methods of treatment. Surely this is an inappropriate use of precious resources. I reject the argument that "It all comes out of the same taxpayers' pockets." I am saying that the job to be done, producing a nontoxic effluent, will be done best and most economically if the role of government is limited to the use of the "carrot and stick."

One of the commenters has identified a number of areas where additional human resources (organic chemists) are required. Surely it would be the rare scientist who could not justify a need for more manpower in his area of interest and responsibility. (Nor would the requests for "more please" cease if these first-generation requests all were filled.) Priorities are not and cannot be set in this way. If we lack an organic chemist working on oil spills, West Coast, it is because this received a lower priority than any of the other 1206 positions assigned to the Fisheries and Marine Service (FMS) in the Pacific region. The opportunity for reordering such priorities within DFE has never been better. Currently the Department is undergoing a zero-base budget review in which all activities must be justified as continuing activities. Hopefully this exercise will identify low-priority programs and lead to their demise or diminution to free resources for new activities with higher priorities.

The FRB background studies were intended as strategy and policy documents. Tactical decisions, such as the transfer of a man-year from one activity to another, for example from surveillance to organic chemistry, are simply an inappropriate level of detail in such national overviews.

The meek may inherit a dearth — I contend that the protection of our environment is limited not so much by the weakness of our understanding, as by our lack of resolve to use the knowledge we have. Examples of this weakness of our resolve are many, but a look at the pulp and paper industry in Ontario will do. During the period 1966–71 there were 12 convictions under the Ontario Water Resources Act, for all such companies in the province, with an average fine of $812.50 — presumably paid out of petty cash. Donnan and Victor (1976) said it all when they wrote:

"It is essential to realize that, in the present economic and legal context, companies have a powerful financial incentive to continue polluting the air and water. . . Fines of this magnitude provide the companies with virtually no economic incentive to incur the much greater costs for pollution control."

In the subsequent 6 years there has not been a single prosecution against any of the 31 companies, despite BOD discharge of 860 tons/day, some 4½ times the objective of 153 tons/day. (In fairness, at time of writing, charges are pending against three companies.)

In 1956 the Ontario Ministry of Environment set water quality objectives for the industry. The first stage, to be met by the end of 1966, required plants to reduce suspended solids to levels deemed appropriate in each case. The second stage, to be met by the end of 1969, required the removal of toxic substances — substances imparting taste to water or fish, color to water, producing foam, etc. Compliance was not achieved by either deadline. Thorough evaluations were conducted in 1970 and in 1975 to measure the effectiveness of the control attempts. The results show that only 3 of 31 mills met the requirement for oxygen demand and only 16 mills met that for suspended solids. In more than a third of the mills, effluent conditions became worse, from 1970 to 1975. Four plants have been identified by the International Joint Commission (IJC) as being potentially hazardous to human health and five more identified as responsible for gross pollution of the environment.

How did we arrive at this sorry state? The strategy was to set nonbinding objectives, seek compliance with each individual mill, forego prosecution during the life of the program, and extend control orders as desired. The mill was thus able to discharge waste freed of the threat of prosecution. When Donnan and Victor noted "This approach has met with only limited success in achieving effluent control by the pulp and paper industry," they were being very kind.

The question that we have to ask is, How much more do we need to know to protect the aquatic environment from the pulp and paper industry in Ontario? I believe the answer to be, "Not much." Or perhaps the question should be, To what avail do we gather more information, if we lack the intestinal fortitude to use it?

The example of pulp and paper in Ontario was chosen because it was well documented; worse instances often are not. (We are indebted to the Ontario Government for releasing this report.)

The federal stance is no less quixotic. The federal Fisheries Act has long been touted as the legal mechanism for protecting the aquatic environment. (Responsibility for enforcing this Act in freshwater has been delegated in part to six provincial governments.) The wording of the Act is clear enough, ". . . no person shall deposit or permit the deposit of a deleterious substance of any type in water frequented by fish . . .," and the penalty is clear enough, "Any person who violates any provision of this section is guilty of an offense and liable on summary conviction to a fine not exceeding five thousand dollars for each offense." Among federal people, there is a belief that the mere threat to invoke the Act will induce/intimidate polluters to comply with environmental objectives. But actual prosecutions under the Act number perhaps a half-dozen annually for all of Canada. Who is the bluffer and who the bluffee?

Weak position, strong troops — During the course of the FRB-sponsored study, I was reminded frequently of something I had once read.[1] Defence of the environment in Canada too often has taken the form of a weak political position defended by strong researchers and managers. This approach is both precarious and costly. I do not fault the people in monitoring, in toxicology, or water chemistry, or whatever, for saddling us with this approach. It is much more widespread than that, especially as a federal strategy. That is, state a weak position and employ a legion of bureaucrats to try to make a purse out of the pig's ear. Perhaps one of the best examples is the Ministry of State for Science and Technology (MOSST) mandate to promote the Make-or-Buy Policy. Would it not have been far less labor-intensive simply to state how much of the scientific activity of each federal department should be done in the private sector, and establish a schedule for achieving this? The existing science line-operation automatically would have made the most effective use of funds earmarked for the private and public sectors. The private sector would have had some insight as to what the future held and could have planned accordingly. Logically the MOSST role in Make-or-Buy Policy should have been put out to the private sector, under Make-or-Buy. But to interlard a layer of program-bending bureaucrats into existing bureaucracies to modify the pattern of federal spending is analogous to using rabbits to guard the lettuce.

In the opening paragraph, reference was made to the "strong troops" guarding the environment. For 1977–78, DFE has an authorized complement of 12,226 man-years, with 3098 identified as scientific and professional, 4297 technical, 2150 administrative support, 1441 operational, 1159 administrative plus foreign service, and 81 executive (Estimates 1977). It is not easy to apportion accurately these people to defense of the environment. The Aquatic Environmental Quality (AEQ) Steering Committee attempted to do this for resources expended in 1976–77 (AEQ Report 1977). Some 1800 man-years were identified as AEQ, comprising 43% of the man-years of Fisheries and Marine Service (FMS), 35% of Environmental Management Service (EMS), and 21% for Environmental Protection Service (EPS). The associated financial commitment to AEQ was $65 million or 13% of the Department total, 1976–77. (For 1977–78 the DFE budget is $545.8 million.) AEQ spending accounted for 44% of the EMS budget, 36% of the FMS, and 18% of the EPS budgets. In addition to this federal effort, there are large dollar and man-year commitments by provincial governments and the private sector, plus lesser amounts by universities and the nonprofit sector.

It is not possible to gauge the adequacy of our environmental protection simply on the basis of human resources employed and dollars expended. Nor is it possible to compare effort in the aquatic area in Canada with that elsewhere. The Canadian responsibility obviously is very large as a consequence of having so much freshwater, the longest coastline, so many boundary waters, extremes of population distribution, extreme demophoric growth, etc. However, it is possible to say that the federal commitment of 1800 man-years to problems of the quality of the aquatic environment is a very large one, that the level of expertise is very high, most workers are housed in new, well-equipped laboratories, and support is adequate if not plush.

If these resources are not accomplishing the job that we believe they should, then we are faced with three choices: (1) to accept a lesser level of environmental protection; (2) to ask for more resources; (3) to make better use of our existing resources.

The first alternative will be opposed by people concerned with the environment and with human health. The second alternative is largely blocked at the political level, both federally and provincially; that is, all spending by government is being constrained in response to public pressure and

[1]When Sixth Army was surrounded at Stalingrad, the Reichsführer declared that henceforth it would be known as "The Fortress Stalingrad." An impertinent junior officer pointed out that a fortress is a strong position maintained by weak troops, and that Stalingrad was the opposite.

the general weakness of the Canadian economy. The third alternative includes looking at strategies that are less labor and capital intensive. First among these is a strategy that says, "You can't put that stuff in the water until you demonstrate that it is harmless." Another strategy is to press on with the development of low-effluent technologies; in some areas this ultimately may be cheaper than current technologies with the associated cost of research, continuous monitoring, new legislation, environmental damage, etc. Another strategy is to make use of the legal mechanisms currently available but largely dormant. Alternatively, if class-action suites were legal in Canada, it would be possible for those who wish to protect the environment through due process of law to supplant the authority of those who do not. We could save on our resources also if we could agree on what are the federal and what are the provincial areas of responsibility in freshwater and the coastal zone; the British North America Act is ludicrously out of date and its ambiguity leads to duplication of effort and an inordinate amount of effort spent in marking territory.

There remains yet another strategy for protecting the environment. In ancient China, doctors were paid only when their patients were well, with disease recognized as evidence of their inadequacy. Perhaps those charged with the responsibility of protecting the environment should be paid, not for diagnosing malaise, or effecting cures, but rather when the environment is maintained in good health. — HAROLD H. HARVEY, *Department of Fisheries and the Environment, Fisheries and Marine Service, Oceanography Branch, Ottawa, Ont. K1A 0E6.*

Acknowledgments — The author is indebted to J. R. Vallentyne, H. A. Regier and F. P. Maher for reading this manuscript.

A GUIDE TO THE OCEAN DUMPING CONTROL ACT. 1975. Fisheries and Marine Service, Ottawa. 9 p.

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Marine Recreational Fisheries. *Edited by Henry Clepper. Sport Fishing Institute, Washington, D.C., 1976. vi + 174 p.*

This book consists of a series of papers presented at the First Annual Marine Recreational Fisheries Symposium held in New Orleans, Louisiana, February 27, 1976. The symposium was sponsored by the International Game Fish Association, the National Coalition for Marine Conservation, and the Sport Fishing Institute. It was supported by the U.S. National Oceanographic and Atmospheric Administration and the National Marine Fisheries Service. In his introduction R. H. Stroud identified the symposium's objectives as follows: (1) to achieve recognition of marine recreational fisheries as an important element of national policy, (2) to identify major recreational fisheries problems and develop fresh solutions thereto, and (3) to foster effective management regimes for the conservation of living marine resources. It couldn't be expected that these objectives could be met at one sitting and presumably that is why the symposium is billed as being the first of an annual series.

It was organized as a series of panels to follow the keynote address by R. M. White. The papers were summarized in a final report by J. C. Stevenson. The panels dealt with four aspects of marine recreational fishing: economic values, social values, management criteria, and institutional functions and responsibilities. To appreciate fully the complexity of the symposium's topic (marine recreational fishing), which is difficult enough to quantify and evaluate even under stable conditions, it should be kept in mind that the national and international background for the symposium was changing rapidly. The U.S. House of Representatives and Senate had just passed legislation extending U.S. fisheries jurisdiction to 200 miles; the U.S. was actively engaged in negotiation at the Law of the Sea Conferences; the old Bureau of Commercial Fisheries and the Bureau of Sport Fisheries (with a long tradition of conflict) had recently been joined into the National Marine Fisheries Service, and the recently developed National Plan for Marine Fisheries had proposed the establishment of Regional Fisheries Management Councils. These Councils (since established) would cut across traditional lines of communication and negotiation between states, between fisheries users and governments, and between commercial and recreational fisheries. Concern with the rapidly changing scene is evident in the papers presented by the panels.

If the background to the symposium was confused by disruption of traditional fisheries structures, the foreground was little less chaotic after the economic panel had presented its views. The problems of assessing in dollar terms, an activity characterized by a large component of personal intangibles, can be illustrated by the wide range of dollar values variously ascribed to the marine recreational fishery — 300 million–7 billion. The panel examined critically the evaluation methods that have been used in the past, the implications of public fishery resource allocation on national income distribution, and future trends in recreational fishing. To the uninitiated (such as this reviewer) it appears that the greatest beneficiaries of a universal licensing system would be the economists, whose most apparent need seems to be a firm data base.

The panel dealing with social values attempted to fit marine recreational activities into current models of human behavior and organization. It made the point that, because a large part of recreational fishing activity consists of personal satisfactions which provide relief from the stresses of modern society, social values must be accounted for in allocation of this (public) resource. Mechanisms for incorporating social values into allocation decisions were proposed and discussed.

The panel on management criteria showed clearly the need for a concerted national policy for marine recreational fishing: the activity itself is proliferating faster than the national population; the nearshore areas which support some of the stocks are becoming polluted; the sport catch for some species now exceeds the commercial harvest; basic biological knowledge needed for optimum sustained yield estimates (abundance, migrations) is lacking for some species; and the states' regulatory efforts are uncoordinated and, in fact, often at cross-purposes. It is clear that pollution abatement, better statistics (obtainable perhaps through licensing), more research, and greater coordination by the federal government are needed to maintain the resources at productive levels and to provide reasonable opportunities for the U.S. public to participate.

The panel on institutional responsibilities, defining as it did present responsibilities of the U.S. federal government and the states, dealt afterward with the mechanisms by which the government can coordinate and support the actions of state governments toward rational development of the marine recreational fishery. The need to manage this fishery in a positive way to satisfy national social and economic needs was clear. Progress has already been made in the form of the Regional Councils, to which the states can be expected gradually and grudingly to give up some of their former sovereignty.

In this reviewer's opinion (himself a "marine recreationist") this volume will *not* bring about a widespread "... recognition of marine recreational fisheries as an important element of (U.S.) national policy ..." because it will not appeal to, nor be read with interest by, large numbers of the recreational fraternity. It is too technical for ready consumption by laymen. Probably it was aimed at the institutional audience; for that it is well-suited since it deals with

technical evaluations comprehensible to senior fishery managers and fisheries academics. Its chief value is that it is a timely review of the current marine recreational fisheries condition, set in the context of rapid change in U.S. fisheries policies. It is rewarding to see how the traditional institutions can and are mobilizing to rationalize the nation's use of its fisheries resource, including the mushrooming recreational fishery. There are lessons in this series of papers for fisheries managers elsewhere, notably in Canada.

The summary by J. C. Stevenson at the end of the volume is competent and can be used as a ready guide to particular papers by the reader interested in special subjects. The print is easy to read and the editing harsh enough to weed out fluff.

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Assessment of the Arctic Marine Environment/ Selected Topics. *Edited by D. W. Hood and D. C. Burrell. Occasional Publication Institute of Marine Science, University of Alaska, Fairbanks, Alaska, 1976. xi + 468 p. $25.00.*

The contents of and rationale for this book are described as "..based on topics selected from a symposium held 11–15 August 1975 in Fairbanks, Alaska, in connection with the Third International Conference on Port and Ocean Engineering under Arctic Conditions (POAC — 75). The official proceedings of the conference, consisting of 108 papers exclusive of the contents of this book, have been compiled separately in two volumes available from the same publisher (Institute of Marine Science, University of Alaska, Fairbanks). The reader is referred to the POAC — 75 proceedings document for discussion of such engineering and mechanical–physical aspects as ice dynamics and related problems of arctic offshore structures and technology, transportation, the seabed and sub-bottom investigations."

As a Symposium collection, the book suffers from the usual problems of topic diversity, nonuniformity of style, and spotty coverage of the title subject. In addition, most of the new information reported on is Alaskan and the focus is the Bering and Beaufort seas, giving the book a strong regional flavor.

The first four chapters are introductory and tread familiar ground, although Dunbar's comments on arctic native people and bureaucratic pollution are worth noting. The second section, six chapters entitled "Regional Program Perspectives", again contains little new information on the North Sea, Soviet arctic research, or Alaskan waters. Hood's review of the Port Valdez marine environment provides a good overview of the trans-Alaskan pipeline terminus.

Sections three (The Northern Seabed Condition) and four (Dynamic Physical Processes) are a hodgepodge of nine chapters dealing with sediments, currents, and waves. The papers are very uneven, ranging from trivial to very good, and contain much of the meat of the book.

Section five, five chapters on Biological Features, is typical of the random coverage of topics elsewhere in the volume. "Primary productivity of sea-ice algae" by Clasby et al. is a low-key initial attempt at quantifying this most important unknown component to arctic marine systems, but no information on other primary producers is given. The shallow-water benthic fauna of Prudhoe Bay is discussed by Feder and Schamel, but there is no discussion of offshore fauna. Craig and McCart's review of anadromous fish between the Mackenzie and Barrow is a useful summary of a resource about which there was almost no information until recently. The paper suffers somewhat in that no reference or comparison is made with eastern arctic anadromous species, but does serve to emphasize what a long way there is to go before the controls on those arctic fish populations are known. The paper by Atkinson, "Development and potential yield of arctic fisheries", is little more than a list of potentially exploitable species and has little application to areas north and east of the Bering Sea. Estimates of yield and discussion of harvest strategy, types of fishery (domestic, commercial, or sports), and a realistic assessment of previous commercial ventures in the arctic marine environment, particularly for char, are absent in this armchair overview. Notably absent in the section on Biological Features are discussions of birds, mammals, most of the benthos, zooplankton, and nutrient dynamics.

Of the four papers in the last section, Hydrocarbons in the Arctic Environment, McAuliffe's paper, "Surveillance of the marine environment for petroleum hydrocarbons", is an excellent overview of the various natural sources of hydrocarbons to the world oceans and the general insignificance of man's contribution. Gosink's discussion of "Sample size in environmental assessments" is excellent even though the title is misleading. The paper deals only with the volume of water samples taken for trace element or particulate analyses.

For each reader there will be bits of interest in the book, even if it is not a total "Assessment of the arctic marine environment". But no matter. There *is* an important message emphasized by two authorities on arctic research, which I quote here in part. Hood (p. 466) says of the negative influence of bureaucracy upon science, "A host of bureaucratic syndromes ... have run the cost of experimentation ... to dangerously high levels. ... For the very nature of bureaucracy is to enhance itself ... until the output or work done by the system is minimal. ... We seem to be more caught up in this nonproductive engulfing tendency in the North than elsewhere, because it becomes the obligation of the few expert workers in

the Arctic to inform the would-be northern experts of the bureaucracy from areas south ...".

Dunbar (p. 19) conveys a similar message on a page entitled "Bureaucratic Pollution": "The growth of this administrative monster continues daily ... and ... this burden ... has handicapped, perhaps more than anything else, the progress in ecological science in the arctic, of which we stand so desperately in need today. ... We are heavily dependent on government science in northern research, ... and it is in government science that this problem has become overwhelming."

Those members of the Canadian civil service, where only the bottom third of the organization gets its feet wet, will probably understand the message.

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(R157)

Aquatic Applications of Ozone. *Edited by Walter J. Blogoslawski and Rip G. Rice. The International Ozone Institute, Inc. Syracuse, N.Y., 1975. viii + 226 p.*

The papers presented in this book give a good perspective of the state of ozone technology in aquatic applications at the time of the workshop. Most of the papers are directly or indirectly related to holding or culturing aquatic organisms with some directed to the use of ozone as a replacement for chlorine as a condenser antifouling agent.

It is, however, somewhat dismaying to see that much of the work has ignored a large body of existing research data on mass transfer, dynamics of disinfection of microorganisms, and the toxicity of ozone to higher as well as lower life forms. The lack of quantification of ozone doses and residual levels is evident in many reports. In fairness, measurement of ozone is difficult, especially in seawater, but again the existing literature on generation of secondary oxidizing molecules by a primary oxidant has apparently not been consulted.

A paper exemplifying the lack of a well-planned and quantified approach described the use of ozone to disinfect water for culturing rainbow trout. Deaths and gill damage were attributed not to ozone toxicity but rather to gas-bubble disease, despite the fact that oxygen levels could at most have reached 105% of saturation. Oxygen supersaturation at this level does not cause gas-bubble disease.

Papers were presented on the successful use of ozone in fishholding, depuration of oysters, and holding of aquatic mammals in aquaria, thus demonstrating the promise and successes of ozone technology.

The book should be very useful for anyone considering research and development using ozone for fish holding or fish culture. It must be read critically with the understanding that the technology is not yet in a state where it can be routinely applied.

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(R131)

Retinas of Fishes. An Atlas. *M. A. Ali and M. Anctil. Springer-Verlag, Berlin, Heidelberg, New York, 1976. 284 + xiii p., 364 figs. $49.20.*

This is an annotated atlas of the microscopic structure of fish retinas, based largely upon specimens collected by the authors and supplemented with illustrations contributed by over a dozen friends and colleagues. The main body of the book comprises: (1) short texts which summarize taxonomic position, habitat and habits, retinal structure in selected species, selected references, and sometimes data on visual pigments; and (2) black-and-white photomicrographs of histological sections accompanied by very brief legends. About two thirds of the 150 families discussed are illustrated.

The authors' intention was to assemble and arrange phylogenetically the otherwise scattered observations on fish retinas, and thereby both to illustrate the variations in retinal structure that relate to the evolutionary diversification of fish species and to guide potential investigators in their search for the most suitable experimental subjects. This ambitious and timely undertaking has produced a handsome and uncluttered volume. The illustrations are well reproduced on heavy, glossy paper and are accompanied by description in large type. A list of fish families as well as a conventional index of genera, species, common names, and significant structures will allow easy access to information. The bibliography is extensive.

The prospective reader should be cautioned that this is a very *general* survey, best suited to giving the novice a general impression of the varied adaptations of retinal form, or to make available to the more advanced student a body of information, particularly on deep-sea and exotic forms, which might otherwise be unavailable in most libraries. In many particulars, however, this is a disappointing and frustrating work. Emptying the shelves of specimens accumulated over a long time and under varying circumstances has produced histological sections and illustrations of inconsistent quality. Modern methods of preparation derived from procedures for electron microscopy are described, but little used. The few illustrations of cells stained selectively by the Golgi method serve only to emphasize how much remains to be done by this and other methods for typing and connectivity analysis of individual neurons. Details of preparation or microscopy for the micrographs are consistently absent.

The textual descriptions of retinal structure will satisfy almost no one. The treatments of groups on

which the authors have already published (e.g. salmonids, pikeperches) are understandably longer and more detailed than others. Otherwise one must go to the original publications by other authors. Descriptions are uncritical, vague, and misleading. Never, for example, do the authors discuss the substantial difficulties in determining whether a retina contains only rods, how many layers or types of a given cell class are present, whether all cells in the "amacrine cell layer" are amacrines, etc. The summaries of "fact" in this atlas, presented without justification, attribution, or scrutiny, can only lead the newcomer to erroneous conclusions.

The literature citations are extensive but incomplete. Unaccountably, the classic 19th century works of (among others) Heinrich Müller on perch, Wilhelm Müller on lamprey, Reich and others on pike, and Krause and Schiefferdecker on just about everything, are totally ignored. Numerous recent works, too, are mysteriously absent: Copeland on vascular patterns, Scholes and Morris on cyprinid bipolar cell connectivity, Naka on catfish neurons, Hollyfield on *Fundulus* embryology, Ehinger and Dowling on interplexiform cells, and Witkovsky on efferent fibers, to name a few.

In summary, this is a book that occupies a unique niche and which, in consequence, a few libraries and investigators concerned with the ecology, ethology, and sensory biology of fishes may wish to have. It is, unfortunately, not the best source for detailed analytical morphology, structure-function correlation, or critical scholarship; nor is it the clearest introduction to retinal laminar organization or visual cell mosaic patterns, nor a comprehensive guide to the literature. It is simply, the only nearly comprehensive survey of fish retinas in print. Prospective readers may well wonder whether so overproduced and underfed a volume is worth the price.

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(R155)

Coastal Zone: Framework for Management in Atlantic Canada. *Douglas M. Johnstone, A. Paul Pross, and Ian McDougal, with special assistance of Norman G. Dale. Institute of Public Affairs, Dalhousie University, Halifax, 1975. xiii + 248 p. (including appendices). $10.00.*

This report, commissioned by the Inland Waters Directorate, Environmental Management Service, in the former Canada Department of the Environment (not Department of Fisheries and the Environment), is an orderly review of the problems generating interest in Coastal Zone Management (CZM). The existing jurisdictions implicated in Atlantic Canadian CZM are reviewed with case history studies illustrating the complexity of the problems. The report concludes by presenting some recommendations for action.

The style as well as the content of the report reflect the difficulties inherent in Coastal Zone Management. However, while the reading is not easy, the report should be of value to those concerned with CZM in areas distant from the target area, Atlantic Canada, particularly persons who regard CZM as a panacea for all the present ills of coastal fisheries.

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(R98)

COMING NEXT MONTH / À PARAÎTRE LE MOIS PROCHAIN

Vertical and seasonal distribution of chlorophyll *a* in Lake Michigan
A. S. Brooks and B. G. Torke

A new digital hydroacoustic data processor and some observations on herring in Alaska
R. E. Thorne

Phytoplankton summer standing crop and annual productivity as functions of phosphorus loading and various physical factors **R. T. Oglesby**

Relationships of fish yield to lake phytoplankton standing crop, production, and morphoedaphic factors **R. T. Oglesby**

Relative toxicity of free cyanide and dissolved sulfide forms to the fathead minnow (*Pimephales promelas*) **S. J. Broderius, L. L. Smith Jr., and D. T. Lind**

Investigation of the impact of mercury on enclosed water columns using a zooplankton simulation model **N. C. Sonntag and W. Greve**

Spawning time and egg size of Atlantic mackerel, *Scomber scombrus*, in relation to the plankton **D. M. Ware**

Temperature and water velocity effects on the swimming performances of young-of-the-year striped mullet (*Mugil cephalus*), spot (*Leiostomus xanthurus*), and pinfish (*Lagodon rhomboides*) **R. A. Rulifson**

Artificial hybrids between the Amur pike *Esox reicherti* and North American esocids
E. J. Crossman and J. W. Meade

Tests for association of transferrin and lactate dehydrogenase phenotypes with weight gain in rainbow trout (*Salmo gairdneri*) **G. L. Reinitz**

Structure of pelagic food chain and relationship between plankton and fish production
R. W. Sheldon, W. H. Sutcliffe Jr., and M. A. Paranjape

A new chironomid genus and species and the immatures of *Baeoctenus bicolor* Sæther (Diptera: Chironomidae) **O. A. Sæther**

Biology of the morid fish *Antimora rostrata* in the western North Atlantic
C. A. Wenner and J. A. Musick

Migrations and activity levels of overwintering Atlantic herring (*Clupea harengus harengus*) along southwest Newfoundland **G. H. Winters**

Storage life of refrozen silver hake (*Merluccius bilinearis*) processed as fillets and minced flesh from thawed, stored, round-frozen fish **D. F. Hiltz, D. H. North, B. S. Lall, and R. A. Keith**

Simulation concepts for fishery systems **L. E. Gales, J. A. Buss, and L. J. Bledsoe**

Grazing of marine copepod developmental stages on naturally occurring particles **S. A. Poulet**

Changes in specific gravity of Atlantic salmon (*Salmo salar*) alevins
R. H. Peterson and J. L. Metcalfe

Notes

A simple device for retrieving instruments or traps set under ice **M. Busdosh and A. E. Manning**

Healing of wounds and location of tags in Atlantic herring (*Clupea harengus harengus*) released with abdominally inserted magnetic tags **G. H. Winters**

Recent invasion of a red alga (*Bangia atropurpurea*) in Lake Michigan **C. K. Lin and J. L. Blum**

The yeast *Phaffia rhodozyma* as a dietary pigment source for salmonids and crustaceans
E. A. Johnson, D. E. Conklin, and M. J. Lewis

Dinoflagellate toxins as probable cause of an Atlantic herring (*Clupea harengus harengus*) kill, and pteropods as apparent vector **A. W. White**

Evidence for the assumption of independence between gastric emptying rate and swimming activity using Atlantic cod, *Gadus morhua* **A. V. Tyler**

Effects of phosphorus from apatite on development of freshwater communities
E. A. Smith, C. I. Mayfield, and P. T. S. Wong

RECENT PUBLICATIONS / RÉCENTES PUBLICATIONS

The number following each title in each series refers to the establishments listed at the end of the section.

Les nombres qui suivent les titres de chacune des séries désignent les établissements suivants à la fin de la section.

Recent Studies

Until 1970, reprints of these articles had been included in an annual volume of "Studies," composed of two parts. The final binding was the 1969 Studies, Part 2, which included No. 1355. These are no longer bound and distributed. We continue, however, to number and list all articles on investigations published in other periodicals, which henceforth may be obtained in reprint form only from the author or originating establishment.

Récentes études

Jusqu'en 1970, ces articles étaient réimprimés, puis reliés dans un volume annuel d'études, comprenant deux parties. Les études de 1969, partie 2, incluant le n° 1355, ont été les dernières à être reliées. Nous n'en faisons désormais plus la reliure ni la distribution. Nous continuons cependant de numéroter les articles et d'en établir des listes d'études sur des recherches mentionnées dans d'autres périodiques. On peut donc se les procurer, sous leur forme réimprimée seulement, de l'auteur ou du service dépositaire.

2274. McBRIDE, J. R., AND U. H. M. FAGERLUND. 1976. Sex steroids as growth promoters in the cultivation of juvenile coho salmon *Oncorhynchus kisutch*. Proc. 7th Annu. Meet. World. Maricult. Soc. p. 145–161. (2)

2275. PARKER, R. R., AND J. SIBERT. 1975. Studies on a production system using a large volume floating pond. 10th Eur. Symp. Mar. Biol., Ostend, Belgium. p. 457–466. (1)

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2277. SCHINDLER, D. W. 1976. Biogeochemical evolution of phosphorus limitation in nutrient-enriched lakes of the Precambrian Shield, p. 647–664. *In* J. O. Nriagu [ed.] Environmental biogeochemistry. Vol. 2. Metals transfer and ecological mass balances. Ann Arbor Science Publishers, Inc., Ann Arbor, Mich.

2278. HALLIDAY, R. G., AND W. G. DOUBLEDAY. 1976. Catch and effort trends for the finfish resources of the Scotian Shelf and estimates of the maximum sustainable yield of groundfish (except silver hake). Int. Comm. Northwest Atl. Fish. Sel. Pap. 1: 11 p.

2279. BIGG, M. A., AND H. D. FISHER. 1974. The reproductive cycle of the female harbour seal off southeastern Vancouver Island, p. 329–347. *In* R. J. Harrison [ed.] Functional anatomy of marine mammals. Vol. 2. Academic Press, Inc., New York, N.Y.

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2292. TAGUCHI, S. 1976. Relationship between photosynthesis and cell size of marine diatoms. J. Phycol. 12: 185–189. (7)

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Canadian Conference For Fisheries Research
Canadian Society of Environmental Biologists

The thirty-first Annual Meeting of the CCFFR will be held at the Holiday Inn, Kent Street, Ottawa, January 4 and 5, 1978. The Fisheries Research Board and the Fisheries and Marine Service, Department of Fisheries and the Environment, will again sponsor this meeting. The President is J. S. Nelson, University of Alberta, Edmonton, the Secretary is J. C. Stevenson, and the Treasurer is J. Camp, both of the Fisheries and Marine Service, Department of Fisheries and the Environment, Ottawa. Program Chairman is E. J. Crossman, Royal Ontario Museum, Toronto.

The Canadian Society of Environmental Biologists will also meet at the Holiday Inn, Ottawa, January 5 and 6, 1978. The President is F. P. Maher, and the Secretary–Treasurer is R. M. Biette, both of the Ontario Ministry of Natural Resources, Toronto. Program Chairman is J. F. S. Bendell, Department of Zoology, University of British Columbia, Vancouver. Local arrangements for CCFFR and CSEB were made by D. E. McAllister and C. Gruchy, both of the National Museum of Natural Sciences, Ottawa.

Members are encouraged to make their hotel reservations as soon as possible.

CCFFR Program

Tuesday January 3 1900–2100 h

Advance Registration

Wednesday January 4 0900–1200 h

Potential Effects of Current and Future Agricultural Practices on Water Resources and Fisheries

Convenor:
L. Lockhart
Department of Fisheries and the Environment
Fisheries and Marine Service
Freshwater Institute
Winnipeg, Manitoba

Wednesday January 4 1400–1700 h

Research on the Matamek River and Its Tributaries, in Quebec. An Integrated Study Applied to Trout–Salmon Habitat

Convenor:
R. J. Gibson
Oceanographic Institute
Woods Hole, Massachusetts

Thursday January 5 0900–1200 h

Environmental Changes and Their Effect on Fish Populations and Fisheries

Convenor:
W. J. Christie
Ontario Ministry of Natural Resources
Picton, Ontario

Thursday January 5 1400–1700 h

Joint CCFFR/CSEB Contributed Papers Session

Persons intending to present papers should contact the Convenor at the earliest possible date, providing the proposed title and, if possible, an abstract of the paper.

Convenor:
G. Pageau
Department of Biology
University of Montreal
Montreal, Quebec

Thursday January 5 1700–1800 h
CCFFR Business Meeting

CSEB Program

Friday January 6 0900–1200 h

Heavy Metals in the Environment with Particular Reference to Land and Mercury, Lead, Arsenic, and Cadmium

Convenor:
P. Stokes
Institute of Environmental Studies
University of Toronto
Toronto, Ontario

Friday January 6 1400–1700 h

Fish and Wildlife Management Based on Practical Research (tentative title)

Convenor:
D. Euler
Ontario Ministry of Natural Resources
Wildlife Branch
Toronto, Ontario

Conférence canadienne relative à la recherche sur les pêches
Société canadienne des biologistes de l'environnement

La 31e réunion annuelle de la Conférence canadienne relative à la recherche sur les pêches (CCFFR) se tiendra au Holiday Inn, rue Kent, Ottawa, les 4 et 5 janvier 1978. Comme par le passé, cette réunion sera sous le patronage de l'Office des recherches sur les pêcheries et le Service des pêches et de la mer, ministère des Pêches et de l'Environnement. Le président est J. S. Nelson, Université de l'Alberta, Edmonton, le secrétaire J. C. Stevenson et le trésorier J. Camp, tous deux du Service des pêches et de la mer, ministère des Pêches et de l'Environnement, Ottawa. Le président du programme est E. J. Crossman, Royal Ontario Museum, Toronto.

La société canadienne des biologistes de l'environnement (CSEB) se réunira également au Holiday Inn, Ottawa, les 5 et 6 janvier 1978. Le président est F. P. Maher et le secrétaire-trésorier R. M. Biette, tous deux de l'Ontario Ministry of Natural Resources, Toronto. Le président du programme est J. F. S. Bendell, Département de zoologie, Université de la Colombie-Britannique, Vancouver. D. E. McAllister et C. Gruchy, tous deux du Musée national des sciences naturelles, Ottawa, s'occupent de l'organisation locale des deux réunions.

Nous encourageons les membres à retenir leur chambre d'hôtel le plus tôt possible.

Programme de la CCFFR

Mardi, le 3 janvier, 1900–2100 h

Enregistrement d'avance

Mercredi, le 4 janvier, 0900–1200 h

Effets possibles des pratiques agricoles
présentes et futures sur les ressources aquatiques et les pêches

Convocateur:
L. Lockhart
Ministère des Pêches et de l'Environnement
Service des pêches et de la mer
Institut des eaux douces
Winnipeg, Manitoba

Mercredi, le 4 janvier, 1400–1700 h

Recherche sur la rivière Matamek et ses tributaires au Québec.
Application d'une étude intégrée à l'habitat
de la truite et du saumon

Convocateur:
R. J. Gibson
Oceanographic Institute
Woods Hole, Mass.

Jeudi, le 5 janvier, 0900–1200 h

Changements dans l'environnement et leur effet
sur les populations de poissons et les pêches

Convocateur:
W. J. Christie
Ontario Ministry of Natural Resources
Picton, Ontario

Jeudi, le 5 janvier, 1400–1700 h

Séance conjointe CCFFR/CSEB consacrée aux contributions
Les personnes qui ont l'intention de présenter un travail sont priées de communiquer avec le convocateur le plus tôt possible et de lui fournir le titre et, si possible, un résumé de leur contribution.

Convocateur
G. Pageau
Département de biologie
Université de Montréal
Montréal, Québec

Jeudi, le 5 janvier, 1700–1800 h
Séance de travail de la CCFFR

Programme de la CSEB

Vendredi, le 6 janvier, 0900–1200 h

Les métaux lourds dans l'environnement, en particulier
le mercure, le plomb, l'arsénic et le cadmium dans le sol

Convocateur :
P. Stokes
Institute of Environmental Studies
Université de Toronto
Toronto, Ontario

Vendredi, le 6 janvier, 1400–1700 h

Gestion du poisson et de la faune sauvage fondée sur la
recherche pratique (titre provisoire)

Convocateur :
D. Euler
Ontario Ministry of Natural Resources
Wildlife Branch
Toronto, Ontario

BULLETIN 198

Seismic Sea Waves — Tsunamis

T. S. MURTY

This Bulletin is an attempt to synthesize current knowledge of tsunamis. Although it is directed primarily to oceanographers, other disciplines are not excluded. The book deals with phase and amplitude dispersion problems and the "Ursell" parameter, which delineates various regimes of dispersion. The classical Cauchy-Poisson problem is also introduced and the subsequent developments in the field of water-wave generation due to explosions is discussed. Tsunami generation by earthquakes and seismic sources such as volcanic explosions and nuclear explosions is considered. Some related phenomena such as landslides and turbidity currents are also included. The propagation of tsunamis across the oceans is discussed. The influence of refraction, diffraction, and scattering is examined, and the problem of trapping tsunami energy by islands and shoals is examined in detail. The coastal tsunami problems such as forerunner, initial withdrawal of water, secondary undulations, and tsunami bore are included, as well as the influence of resonance on tsunamis. Tsunamis in various parts of the world are described. Tsunami warning systems of the past, present, and future are discussed, as well as tsunami instrumentation and protection measures. Background information on seismology is in the Appendix (in microfiche form).

(French edition in preparation) 337 pages

Ce Bulletin tente de synthétiser nos connaissances actuelles sur les tsunamis. Bien que préparé surtout à l'intention des océanographes, il n'exclut pas les autres disciplines. Le volume traite des problèmes de dispersion de phase et d'amplitude, et du paramètre d'«Ursell», qui définit les divers régimes de dispersion. On introduit également le problème classique de Cauchy-Poisson ainsi que les développements qui se sont produits par la suite dans le domaine de la génération des vagues causées par des explosions. Nous examinons la formation de tsunamis par tremblements de terre et à la suite de phénomènes séismiques tels qu'éruptions volcaniques et explosions nucléaires. On y inclut certains phénomènes connexes tels que les glissements de terrain et les courants de turbidité. On discute de la propagation des tsunamis dans les océans. L'influence de la réfraction, de la diffraction et de la dispersion est étudiée, de même que le problème du harnachement de l'énergie des tsunamis par les îles et les hauts-fonds, qui est examiné en détail. Ce Bulletin couvre également les problèmes des tsunamis côtiers, entre autres l'onde montante, le retrait initial de l'eau, les ondulations secondaires, les mascarets, ainsi que l'influence de la résonnance sur les tsunamis. Nous décrivons les tsunamis dans diverses parties du monde. Nous discutons des systèmes d'alerte passés, présents et futurs, de même que l'instrumentation et les mesures de protection relatives aux tsunamis. L'Annexe (sous forme de micro-fiche) contient une information de base sur la séismologie.

(La version française est en préparation)

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